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# ADAMTS-7 Inhibits Re-Endothelialization of Injured Arteries and Promotes Vascular Remodeling Via Cleavage of Thrombospondin-1

**Running title:** *Kessler et al.; ADAMTS-7 inhibits re-endothelialization via TSP-1*

Thorsten Kessler, MD[1,*]; Lu Zhang, PhD[2,*]; Ziyi Liu, PhD[2]; Xiaoke Yin, PhD[3]; Yaqian Huang, PhD[2]; Yingbao Wang, BS[2]; Yi Fu, PhD[2]; Manuel Mayr, MD, PhD[3]; Qing Ge, MD, PhD[4]; Qingbo Xu, MD, PhD[3]; Yi Zhu, MD[5]; Xian Wang, MD, PhD[2]; Kjestine Schmidt, PhD[6,7]; Cor de Wit, MD[6,7]; Jeanette Erdmann, PhD[7,8]; Heribert Schunkert, MD[1,9]; Zouhair Aherrahrou, PhD[7,8,*]; Wei Kong, MD, PhD[2,*]

for the German Mouse Clinic Consortium[**]

[1]Deutsches Herzzentrum München, Klinik für Herz- und Kreislauferkrankungen, Technische Universität München, München, Germany; [2]Dept of Physiology and Pathophysiology, School of Basic Medical Sciences, Peking University; Key Laboratory of Molecular Cardiovascular Science, Ministry of Education, Beijing, China; [3]Cardiovascular Division, Kings College London BHF Centre, London, United Kingdom; [4]Dept of Immunology, School of Basic Medical Sciences, Peking University, Beijing, China; [5]School of Basic Medical Sciences, Tianjin University, Beijing, China; [6]Institut für Physiologie, Universität zu Lübeck, Lübeck, Germany; [7]Deutsches Zentrum für Herz-Kreislauf-Forschung (DZHK) e.V. (German Center for Cardiovascular Research), partner site Hamburg/Kiel/Lübeck, Lübeck, Germany; [8]Institut für Integrative und Experimentelle Genomik, Universität zu Lübeck, Lübeck, Germany; [9]Deutsches Zentrum für Herz-Kreislauf-Forschung (DZHK) e.V. (German Center for Cardiovascular Research), partner site Munich Heart Alliance (MHA), München, Germany

[*]These authors contributed equally to this work.

[**]The members of the German Mouse Clinic Consortium are listed in the Supplemental Material

**Address for Correspondence:**

Wei Kong, MD, PhD
Dept of Physiology and Pathophysiology
Basic Medical College of Peking University
Beijing 100191
People's Republic of China
Tel: +86 10 82805594
Fax: +86 10 82805594
E-mail: kongw@bjmu.edu.cn

Zouhair Aherrahrou, PhD
Institut für Integrative und Experimentelle Genomik
Universität zu Lübeck
Maria-Goeppert-Str. 1, 23562 Lübeck,
Germany
Tel: +49 451 500 3392
Fax: +49 451 500 5767
E-mail:zouhair.aherrahrou@iieg.uni-luebeck.de

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## Abstract

***Background*—**ADAMTS-7, a member of the disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family, is recently identified to be genome-wide significantly associated with coronary artery disease (CAD). However, the mechanisms that link ADAMTS-7 and CAD risk remain elusive. We have previously demonstrated that ADAMTS-7 promotes vascular smooth muscle cell migration and post-injury neointima formation via degradation of a matrix protein cartilage oligomeric matrix protein (COMP). Because delayed endothelium repair renders neointima and atherosclerosis plaque formation after vessel injury, we examined whether ADAMTS-7 also inhibits re-endothelialization.

***Methods and Results*—**Wire-injury of the carotid artery and Evans blue staining were performed in *Adamts7*$^{-/-}$ and wildtype mice. Adamts-7 deficiency greatly promoted re-endothelialization at 3, 5, and 7 days after injury. Consequently, Adamts-7 deficiency substantially ameliorated neointima formation in mice at days 14 and 28 after injury compared with the wildtype. *In vitro* studies further indicated that ADAMTS-7 inhibited both endothelial cell proliferation and migration. Surprisingly, COMP deficiency did not affect endothelial cell proliferation/migration and re-endothelialization in mice. In a further examination of other potential vascular substrates of ADAMTS-7, a label-free LC MS/MS secretome analysis revealed thrombospondin-1 (TSP-1) as a potential ADAMTS-7 target. The subsequent studies showed that ADAMTS-7 was directly associated with TSP-1 by its C-terminus and degraded TSP-1 *in vivo* and *in vitro*. The inhibitory effect of ADAMTS-7 on post-injury endothelium recovery was circumvented in *Tsp1*$^{-/-}$ mice.

***Conclusions*—**Our study revealed a novel mechanism by which ADAMTS-7 affects neointima formation. Thus, ADAMTS-7 is a promising treatment target for post-injury vascular intima hyperplasia.


**Key words:** vascular remodeling, re-endothelialization, metalloproteinase, neointima formation



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## Introduction

Endothelial cells (EC) play an essential role in the modulation of vascular homeostasis. During aging and specifically during the development of atherosclerosis, endothelial cells are exposed to various damaging stimuli and are thereby prone to injury[1]. Rapid endothelial recovery, or re-endothelialization, correlates with diminished plaque formation[2]. Likewise, coronary intervention-induced vascular injury requires an efficient re-endothelialization to prevent post-injury restenosis and thrombotic events. The rate of luminal endothelial repair is thus a critical modulator of arterial lesion formation after injury. Drug-eluting stents have failed to improve the long-term prognosis and increased the stent thrombosis rate [3-5] potentially because they inhibit not only vascular smooth muscle cell (VSMC) proliferation/migration but also re-endothelialization[6, 7]. Therefore, new strategies that aim to promote endothelial recovery, as well as simultaneously inhibit VSMC activation are needed for the effective prevention and treatment of atherosclerosis and post-injury restenosis.

Metalloproteinases are critical in vascular wall remodeling through matrix or non-matrix degradation[8]. Recently, we described ADAMTS-7, a member of a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family, in the mediation of VSMC migration and the promotion of neointima formation following artery injury through degradation of cartilage oligomeric matrix protein (COMP)[9, 10]. To date, COMP is the only identified substrate of ADAMTS-7 in the vessel wall and believed to mediate its functional effects [11, 12]. Three recent genome-wide association studies (GWASs) have further revealed ADAMTS7 as a novel locus associated with human coronary atherosclerosis[13-15]. A non-synonymous SNP (single nucleotide polymorphism) in the prodomain of ADAMTS-7 is inversely related to VSMC migration, COMP cleavage and the prevalence of atherosclerosis[16]. Moreover, ADAMTS-7



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promotes VSMC and aortic calcification by disturbing the balance between osteogenic BMP-2 and its natural inhibitor COMP[12, 17]. However, the underlying mechanism of ADAMTS-7 in atherogenesis and post-injury vascular remodeling remains elusive. In this current study, we report that ADAMTS-7 not only promotes VSMC activation but also inhibits post-injury endothelial cell recovery via a COMP-independent mechanism.

## Methods

All animal studies followed the guidelines of the Animal Care and Use Committees of Peking University, People's Republic of China and Schleswig-Holstein and Bavaria, Germany. The *Adamts7* gene was interrupted by introducing an internal ribosome entry site followed by the beta-galactosidase sequence between exons 4 and 5. Wire-injury of the mouse carotid artery was performed in 12-week-old male mice as described[18]. Gel-LC-MS analysis of secretome was performed as described[19]. An expanded and detailed Materials and Methods section is available in the Online Supplemental Data.

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## Results

### *Adamts7*$^{-/-}$ mice are viable and do not show any obvious phenotype

The *Adamts7* gene was interrupted by the introduction of an internal ribosome entry site followed by the beta-galactosidase sequence between exons 4 and 5 (**Figure 1A**). Interruption of the gene was visualized by PCR on genomic DNA and RT-PCR on mRNA from heart, kidney, and liver tissues (**Figure 1B-C**). Correct introduction of the beta-galactosidase sequence was verified by X-gal staining of heart tissue (**Figure 1D**).

### Large-scale phenotyping of *Adamts7*$^{-/-}$ mice

Male and female *Adamts7*$^{-/-}$ mice were fertile and segregated with the assumed Mendelian



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frequencies. WT- and *Adamts7*$^{-/-}$ mice were subjected to a large-scale phenotyping as previously described[20]. As shown in **Supplemental Table 1-4**, aside from changes in anxiety-related behavior, Adamts7$^{-/-}$ mice did not display abnormal phenotypes measured by vital parameters or echocardiographic analysis or severe organ dysfunction or histopathological abnormalities. Lung function analysis revealed increased lung function parameters and reduced resistance. However, histopathologic analysis did not detect emphysema (data not shown). We did found some sex-specific effect in particular on energy metabolism (**Supplemental Table 2**). However, without a proatherogenic background, *Adamts7*$^{-/-}$ mice did not display changes in blood lipid levels after 15 weeks of western diet compared to WT mice (**Supplemental Table 4**).

**ADAMTS-7 deficiency promotes re-endothelialization and ameliorates neointima formation in wire-injured mouse carotid arteries**

To test the hypothesis if ADAMTS-7 is involved in post-injury endothelium recovery, we performed wire injury in the carotid artery of *Adamts7*$^{-/-}$ mice and littermate wild type C57/BL6 mice. Re-endothelialization was quantified by *en face* Evans blue staining of the denuded area at 3, 5 and 7 days after injury (**Figure 1E**). WT endothelial cells were severely damaged immediately after injury and recovered by approximately 25% at day 3, 45% at day 5 and 65% at day 7. In contrast, re-endothelialization in *Adamts7*$^{-/-}$ mice was 50% at day 3, 75% at day 5 and 85% at day 7 post-injury. Subsequent neointimal hyperplasia was completely abolished in *Adamts7*$^{-/-}$ mice at 14 and 28 days. Of note, media area and circumference of EEL did not differ between the two groups (**Figure 1F**).

**ADAMTS-7 inhibits endothelial cell proliferation *in vivo* and *in vitro***

Next, we examined whether ADAMTS-7 affects EC proliferation. *In vivo* proliferation was assessed by BrdU incorporation. Endothelial cells were identified by *en face*



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immunofluorescence staining of von Willebrand Factor (vWF). The BrdU positive cells by *en face* staining reflected proliferating cells at the wound margins proximal to the impaired artery. Endothelial cell proliferation was significantly enhanced in *Adamts7*$^{-/-}$ mice compared with WT mice 3 days after injury (**Figure 2A**).

Next, we monitored HUVEC proliferation *in vitro*. Ectopic expression of ADAMTS-7 by adenovirus (Ad-*ADAMTS-7*) at 10 multiplicities of infection (MOI) markedly repressed HUVEC proliferation as evidenced by cell counting via kit-8 (CCK-8), by cell cycle analysis via flow cytometry and cell cycle checkpoint protein measurement by Western Blot (**Figure 2 B-D**). A neutralizing antibody that targeted the metalloproteinase domain of ADAMTS-7 was applied and functionally characterized. The antibody circumvented the COMP degradation capacity of ADAMTS-7 in a dose-dependent manner (**Supplemental Figure 1A**), as well as ADAMTS-7 induced VSMC migration (**Supplemental Figure 1B**). Interestingly, the ADAMTS-7 neutralizing antibody dose-dependently reversed the inhibitory effect of ADAMTS-7 on EC proliferation (**Figure 2E**). These data reinforced that ADAMTS-7 specifically targets EC proliferation.

**ADAMTS-7 represses endothelial cell migration**

As cell migration is an essential step in the re-endothelialization response, we assessed the effects of ADAMTS-7 on endothelial cell migratory ability. An *in vitro* scratch-wound assay revealed reduced migration of Ad-*ADAMTS-7*–infected HUVECs compared with Ad-*LacZ*–infected cells. The mean migration distance was shorter compared with the control cells by 48%, 32% and 43% at 12, 18, and 24 hours after injury, respectively (**Figure 3A**). Additionally, modified Boyden chamber assays were performed. A dramatic decrease of migration was observed in Ad-*ADAMTS-7*-infected HUVECs compared with Ad-*LacZ* infected cells (**Figure**



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**3B**). Reciprocally, ADAMTS-7 inhibition by neutralization antibody significantly abolished the anti-migratory effect of ADAMTS-7 (**Figure 3C**).

**COMP does not affect EC proliferation/migration**

Our previous studies have shown that ADAMTS-7 directly binds to and degrades COMP in VSMCs and injured vessels and subsequently promoting VSMC migration[9, 10]. A recent study also revealed an ADAMTS-7 SNP to alter COMP degradation and VSMC migration and therefore affecting coronary artery disease risk[14]. Because COMP is primarily expressed in VSMCs but not in endothelial cells[21], we then examined whether COMP also affects re-endothelialization. Thus, HUVECs were supplemented with increasing amount of purified COMP (concentration from 50 to 200 ng/ml) or cultured on plates coated with purified COMP. Interestingly, neither treatment affected HUVEC proliferation (**Supplemental Figure 2A-B**). In accordance, scratch-wound assays on HUVECs supplemented with various amounts of COMP revealed no difference in migration (**Supplemental Figure 2C**). To avoid the high level of mitogens masking potential effects of COMP, proliferation and migration of HUVEC were analyzed in the presence of 1% FBS. Neither cell proliferation nor migration was influenced by increasing amount of purified COMP (**Supplemental Figure 2D&E**). Next, wire-injury was conducted in *Comp*$^{-/-}$ and WT mice. In accordance, re-endothelialization was not significantly different between WT and *Comp*$^{-/-}$ mice (**Supplemental Figure 2F**). In contrast, the neointima area was greatly increased in *Comp*$^{-/-}$ mice 28 days after wire-injury compared to littermate wild type mice (**Supplemental Figure 2G**), reinforcing the notion that ADAMTS-7 retards endothelium repair independent of COMP. These data suggest that although ADAMTS-7 promotes VSMC migration and neointima formation via COMP degradation, ADAMTS-7 may inhibit EC recovery via COMP-independent mechanisms.



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**Identification of a novel substrate for ADAMTS-7 by secretome analysis**

To identify a novel substrate of ADAMTS-7 and reveal the mechanism of ADAMTS-7 on re-endothelialization, a secretome proteomics analysis was performed. Primary rat VSMCs were adenovirally infected with Ad-*ADAMTS-7* or control Ad-*GFP*. Supernatant was collected and analyzed by proteomics. As expected, ADAMTS-7 was dramatically increased. The gel-LC-MS/MS analysis identified 290 proteins in the conditioned media, of which, 29 proteins were identified with significant differences ($P \leq 0.05$) (**Supplemental Table 5**). Among these, 13 proteins were extracellular proteins or plasma membrane proteins. These proteins included extracellular matrix proteins (Thrombospondin-1, Osteopontin, periostin, Olfactomedin-like protein 3, Growth/differentiation factor 6 and Sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1), cell proliferation regulators (Pigment epithelium-derived factor and Guanine nucleotide-binding protein subunit beta-2-like 1), peroxidases (Thioredoxin), and cytoskeleton proteins (Actin-related protein 3, Elastin, Moesin and Septin-11).

Thrombospondin-1 (TSP-1) is the first identified and potent endogenous anti-angiogenic protein capable of inhibiting EC proliferation and migration and was of close to statistical significance ($P=0.0509$) regulated by ADAMTS-7 overexpression. Further ELISA analysis confirmed the reduced secretion of TSP-1 in the supernatant of Ad-*ADAMTS-7* compared with Ad-*LacZ* infected T/G HA VSMCs (**Figure 4A**). TSP-1 has been shown to be expressed and secreted by ECs[22, 23]. Similarly, the secretion of endogenous TSP-1 from HUVECs was markedly reduced by Ad-*ADAMTS-7* infection compared with Ad-*LacZ* treatment ($4.798 \pm 0.7136$ *vs.* $2.145 \pm 0.8099$ ng/$10^4$ cells; n=8; $P<0.05$) (**Figure 4B**). In addition, increased TSP-1 was detected in the aorta of *Adamts7*$^{-/-}$ mice, which indicates ADAMTS-7 truly affects the TSP-1 level (**Figure 4C**).



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**ADAMTS-7 associates with TSP-1**

As protein-protein interactions are a fundamental process for most enzyme-substrate reactions, we first examined the interaction between ADAMTS-7 and TSP-1. The co-immunoprecipitation (Co-IP) assay was conducted to verify the association of ADAMTS-7 and TSP-1 *in vivo*. In WT aorta, a specific TSP-1 band was present in the complex immunoprecipitated with anti-ADAMTS-7 antibody, but not with control IgG. Accordantly, Co-IP with anti-TSP-1 antibody revealed that TSP-1 also precipitated ADAMTS-7 (**Figure 5A**). In contrast, no protein interaction was detected in *Adamts7*$^{-/-}$ aorta. A specific interaction between ADAMTS-7 and TSP-1 was further confirmed in primary HUVECs, as well as COS-7 cells co-transfected with ADAMTS-7 and TSP-1 (**Figure 5B-C**). To further characterize the binding motif of ADAMTS-7 attributable to the TSP-1 interaction, a mammalian two-hybrid assay was performed by co-transfecting Eahy 926 cells with the pACT plasmids that expressed various ADAMTS-7 deletion mutants and the pBIND plasmid that encoded the full-length TSP-1, respectively. The ADAMTS-7 prodomain (aa 26-246), the metalloproteinase plus disintegrin-like and cysteine-rich domain (aa 238-711), and the spacer-1 plus three TSP repeats (aa 703-1007) were not bound to TSP-1. Instead, the spacer-2 plus four C-terminal TSP repeats of ADAMTS-7 (aa 999-1595) bound to TSP-1 (**Figure 5D**). The interaction between the ADAMTS-7 C- terminus and TSP-1 was also confirmed by Co-IP with anti-Flag antibody in Eahy 926 cells transfected with Flag-CMV vectors that expressed various ADAMTS-7 deletion mutants (**Figure 5E**). Taken together, our data show that ADAMTS-7 binds to TSP-1 *in vitro* and *in vivo*.

**ADAMTS-7 degrades TSP-1 *in vitro***

Next, we analyzed TSP-1 cleavage by ADAMTS-7 in HUVECs. Western blot analysis of Ad-*ADAMTS-7* and Ad-*LacZ* infected cells revealed reduced levels of full-length TSP-1 in both



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whole cell lysate (**Figure 6A**) and supernatant (**Figure 6B**). Interestingly, a 140-kD fragment was repeatedly observed in the supernatant, but not in the cell lysate. In contrast, the mRNA level of TSP-1 was not altered by ADAMTS-7 (**Supplement Figure 3**). To further verify the observation, COS-7 cells were infected with increasing amounts of adenoviral constructs that expressed LacZ and ADAMTS-7, respectively. The supernatant was collected, concentrated and incubated with purified human TSP-1 at 37°C for 4 h. With increased expression of ADAMTS-7, a gradually reduced level of full-length of TSP-1 (170 KD) was observed in parallel with an increased 140-kD fragment (**Figure 6C**). Accordingly, an enhanced cleavage fragment of TSP-1 was observed with increasing amounts of extraneous TSP-1 (**Figure 6D**). This effect, however, was completely abolished by adding the ADAMTS-7 neutralizing antibody (**Figure 6E**). Previous studies have suggested that the catalytic domains of ADAMTS-7 and ADAMTS-20 produced in bacteria can digest their substrates *in vitro*[9]. Using a similar method, we purified the catalytic domain (aa 217– 427) of rADAMTS-7 as a GST-fusion protein in bacteria. The GST moiety was further removed by thrombin, and the purity of protein was confirmed by visualization using Coomassie staining (data not shown). The recombinant catalytic domain of ADAMTS-7 was incubated with purified human TSP-1 in a buffer containing 50 mM Tris-HCl, 150 mM NaCl, 5 mM CaCl 2,2 mM ZnCl2, and 0.05% Brij-35, pH 7.5. As shown in **Figure 6F**, the catalytic domain of ADAMTS-7 digested TSP-1 in a dose-dependent manner.

**ADAMTS-7 inhibits re-endothelialization via TSP-1**

We next examined whether ADAMTS-7 inhibited post-injury EC recovery via TSP-1. TSP-1 expression was specifically silenced by siRNA treatment of HUVECs (**Figure 7A**). As shown in **Figure 7B-C**, the inhibitory effect of ADAMTS-7 on EC proliferation was significantly abolished in the absence of TSP-1 as evident by both cell counting and cell cycle analysis. In line



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with this observation, TSP-1 deficiency also circumvented the inhibitory effect of ADAMTS-7 on EC migration (**Figure 7D**). As a consequence, the retardation of re-endothelialization by ADAMTS-7 overexpression was circumvented in *Tsp1*$^{-/-}$ mice (**Figure 7E**), which indicates ADAMTS-7 refrains endothelial repair from repairing via TSP-1 (**Figure 8**).

## Discussion

Aberrant endothelial cell recovery is inversely related to neointima formation during atherosclerosis and post-injury restenosis. Our current study revealed ADAMTS-7 as a potent inhibitor of endothelial recovery in response to injury. Using *Adamts7*$^{-/-}$ mice and injury models, we uncovered that in addition to the suppression of VSMC migration, ADAMTS-7 deficiency also promoted re-endothelialization and completely blocked subsequent neointima formation. ADAMTS-7 inhibition, therefore, is a promising dual-effect target for both atherosclerosis and restenosis after PCI.

The *ADAMTS-7* locus was identified to have a strong association with coronary atherosclerotic disease[14, 15] and was rather involved in the formation of atherosclerotic plaques compared with atherothrombotic events. However, the underlying mechanism is not yet understood. In the current phenotype screening assay, we did not observe difference regarding lipid metabolism. As shown in **Supplemental Table 4**, without a proatherogenic background, *Adamts7*$^{-/-}$ mice did not display changes in blood lipid levels after 15 weeks of western diet compared to WT mice. However, we did found increased maximum oxygen consumption in male mice (**Supplemental Table 2**), indicating alterations in the energy metabolism or cardiorespiratory fitness during exercise[24]. Further experiments are needed for better understanding of *Adamts7*$^{-/-}$ mice phenotype.



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Previously, we demonstrated that ADAMTS-7 degrades COMP in vessels[10]. COMP itself interacts with α7β1 integrin and BMP-2 and prevents VSMC trans-differentiation into a synthetic or osteogenic phenotype[11, 12]. ADAMTS-7, via degradation of COMP, promotes neointima formation and vascular calcification[10, 17]. Among other potential substrates, we have identified TSP-1 as another ADAMTS-7 target with profound functional implications on endothelial biology following injury. ADAMTS-7 not only acts via COMP degradation but also inhibits EC proliferation/migration via a COMP-independent pathway. TSP-1 belongs to the microcellular thrombospondin protein family, which also includes TSP-2, TSP-3, TSP-4, and TSP-5 (COMP)[25]. Our functional data are in line with previous reports that demonstrates intra-arterial delivery of TSP-1 antibodies accelerates re-endothelialization and reduces neointimal lesion formation after balloon denudation in rats[26]. Our study is also consistent with earlier studies that showed reduced neointima hyperplasia in *Tsp1*$^{-/-}$ mice[27]. Compelling studies have shown the pivotal role of TSP-1 in angiogenesis, inflammation, wound healing, cancer and thrombosis via complex protein-protein interactions with various partners, such as CD47, TGF-β, CD36, and integrin[28]. TSP-1 has further been detected in atherosclerotic specimens, and the genetic variants of TSP-1 have been reported to correlate with CAD and myocardial infarction[29-31]. In *Apoe*$^{-/-}$ mice, TSP-1 deficiency has also been shown to accelerate atherosclerotic plaque maturation without affecting plaque formation[32]. In addition to inhibit endothelial recovery, we further ask whether TSP-1 mediates ADAMTS-7 promotion of VSMCs migration. As shown in **Supplemental Figure 4**, ADAMTS-7 silencing/overexpression induced repression/enhancement of VSMCs migration was not affected by TSP-1 deficiency. In contrast, reduced VSMCs migration by ADAMTS-7 silencing was almost completely rescued by COMP deletion, which is in accordance with our previous study[10], indicating that TSP-1 is not involved in ADAMTS-7



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mediated VSMCs migration.

Compelling evidence indicates TSP-1 was relatively abundant in EC cells or mainly present in endothelium of the stenotic surface of coronary artery[29, 33], although it was identified in almost all layers of injured artery. To address the cellular origin of increased TSP-1 in *Adamts7*$^{-/-}$ aorta, we have further isolated the aortic ECs and VSMCs from *Adamts7*$^{-/-}$ and wild type mice, respectively. Western blot analysis revealed that TSP-1 protein level increased in both ECs and VSMCs from *Adamts7*$^{-/-}$ mice compared to WT mice (**Supplemental Figure 5**). By using ELISA analysis, we revealed about 7-fold basal amount of TSP-1 in the supernatant of EC compared to VSMCs (**Figure 4 A&B**), indicating ADAMTS-7 may preferentially target EC TSP-1. However, we can not exclude the possibility that ADAMTS-7 degrading of TSP-1 from both EC and VSMCs.

Interestingly, both ADAMTS-7 and TSP-1 exhibit potent anti-re-endothelialization effects that cannot simply be explained by reduced expression or secretion of TSP-1 secondary to cleavage by ADAMTS-7. One potential explanation is that TSP-1 cleavage by enhanced ADAMTS-7 leads to the production of a bioactive TSP-1 fragment with a more potent inhibitory effect on EC recovery, as exampled by a previous study that showed ADAMTS-1 mediates the release of anti-angiogenic polypeptides from TSP-1 and TSP-2[34]. After mutating the ADAMTS-1 cleavage site in TSP-1 (glutamic acid 311 and leonine 312) and cell transfection, the degradation of TSP-1 by ADAMTS-7 was still detectable, which indicates different cleavage sites of ADAMTS-7 and ADAMTS-1, and thus highlights the putative different signaling pathway that involve ADAMTS-7 and TSP-1 (**Supplemental Figure 6**). Similarly, bioactive ECM fragments generated by matrix metalloproteinases (MMPs) and cathepsins have been shown to exhibit various effects via novel receptors[35-37]. Further studies are needed to clarify the cleavage site of



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TSP-1 by ADAMTS-7 and the biological function of the cleaved fragment on EC function and vascular repair.

There is other potential possibility of TSP-1 mediated repression of ADAMTS-7 on EC recovery. ADAMTS-7 may release extracellular matrix-bound TSP-1 and therefore activate the latent TSP-1. To address this issue, we tried to analyze the ECM-bound TSP-1 in primary *Adamts7* deficient EC and VSMCs. However, just by regular Western blot analysis we could hardly detect the full-length TSP-1 in the ECM of both EC and VSMCs (data not shown), whereas abundant TSP-1 was observed in the supernatant. By using similar protocol, previous studies also found a few ECM-bound TSP-1[34, 38]. Previous quantitative proteomics analysis has revealed TSP-1 in the ECM of human vessel, but as a less abundant protein compared to other matrix protein such as fibronectin[39]. Further quantitative ECM proteomics analysis in WT and *Adamts7*$^{-/-}$ aorta is needed to explore the possibility that ADAMTS-7 release of ECM-bound silent TSP-1. Nevertheless, compared to low level of TSP-1 in the ECM, greater amount of TSP-1 or fragment in the supernatant may play more important role to EC cells.

In conclusion, ADAMTS-7-mediated TSP-1 cleavage may play an important role in re-endothelialization during human vascular injury-repair response. In addition to its COMP mediated effects, ADAMTS-7 might involve a second target mechanism for the prevention/therapy of vascular neointima hyperplasia.

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**Conflict of Interest Disclosures:** None.

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**Figure Legends:**

**Figure 1.** Adamts-7 deficiency promotes re-endothelialization and ameliorates neointima formation in wire-injured mice carotid arteries. (A) Targeting vector for genomic deletion of *Adamts7*. *Adamts7*$^{-/-}$ embryonic stem cells were used to generate *Adamts7*$^{-/-}$ mice by the insertion of an internal ribosome entry site followed by the *β-Gal* sequence and a neomycin cassette (trans-NIH Knock-Out Mouse Project, KOMP Repository, USA; www.komp.org). (B) Genomic PCR results of the *Adamts7*$^{-/-}$ mice. (C) *Adamts7* gene expression in heart, kidney and liver. (D) Representative pictures of *β*-gal staining of heart tissue. (E) Re-endothelialization was quantified in Evans blue stained carotid arteries at 3, 5 and 7 days after vascular injury (representative



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pictures). Blue staining indicates endothelial denudation. Scale bar, 1 mm, *$P$<0.05 *vs.* wild type (n=6 to 8 for each group). (F) Neointima formation was determined on hematoxylin and eosin–stained cross sections of carotid arteries 14 and 28 days after vascular wire injury (n=6 each group). Scar bar, 100 μm. *$P$<0.05. n.d. indicates not detectable.

**Figure 2.** ADAMTS-7 inhibits HUVEC proliferation *in vivo* and *in vitro.* (A) Representative BrdU positive endothelial cells at the endothelial wound margins in carotid arteries 3 days after vascular injury. Proliferating nuclei were indicated by BrdU incorporation (red), and endothelial cells were indicated by vWF staining (green, n=3 each group). Scar bar, 50 μm. (B) Proliferation of HUVECs infected with Ad-*ADAMTS-7* or Ad-*LacZ* was determined by Cell Counting Kit-8 (CCK-8). Results are means±SEM from 3 independent experiments performed in duplicate. (C) Cell-cycle distribution of HUVECs was confirmed by propidium iodide staining and FACS analysis. Results are means±SEM from 3 independent experiments performed in duplicate. (D) Representative Western blot of cell cycle checkpoint protein in Ad-*ADAMTS-7* and Ad-*LacZ* infected HUVECs. Bar represent means±SEM from 3 independent experiments. (E) ADAMTS-7 inhibition by neutralization antibody abolished the anti-proliferation effect. HUVECs were supplemented with ADAMTS-7 neutralization antibody (0.1-50 μg/ml) in culture medium when infected with adenovirus. Results are means±SEM from 4 independent experiments performed in duplicate.

**Figure 3.** ADAMTS-7 suppresses HUVEC migratory ability *in vitro.* (A) Representative images of cell migration 12 hours after scratching. Confluent HUVEC monolayers were scratch wounded 48 hours after adenoviral infection. The cells were maintained in culture for an



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additional 12, 18 and 24 hours prior to imaging (dotted line indicates wound edge). The mean distance migrated by the HUVECs was quantified (average of 4 independent microscope fields for 3 independent experiments each, magnification ×100). (B) Representative images of modified Boyden chamber assay at 6, 9 and 12 hours (magnification ×100). Migrated cells were quantified by the average of 4 randomly chosen high-power fields (HPF) of 3 independent duplicate experiments. (C) Neutralization antibody (1-50 μg/ml) rescued the suppressed migration in Ad-*ADAMTS-7* infected HUVECs 12 hours after scratch. Results are means±SEM from 3 independent experiments.

**Figure 4.** ADAMTS-7 decreases thrombospondin-1 expression at the protein level. (A) Identification of the TSP-1 protein level in the culture medium of Ad-*lacZ* or Ad-*ADAMTS-7* infected T/G HA VSMC by ELISA analysis. Results are means±SEM from 5 independent experiments. (B) Protein level of TSP-1 in HUVEC supernatant. Results are means±SEM from 8 independent experiments. (C) Western blot analysis of TSP-1 expression in the aorta of *Adamts7*$^{-/-}$ mice. n=3.

**Figure 5.** ADAMTS-7 associates with TSP-1 via its C-terminal domain. TSP-1 was associated with ADAMTS-7 in mouse aorta (A), HUVECs (B) and COS-7 cells co-transfected with TSP-1 and ADAMTS-7 (C) by Co-IP. (D) Upper, schematic illustration of ADAMTS-7 structures used to map the corresponding domains that bind to TSP-1. Presence or absence of binding between ADAMTS-7 and TSP-1 was indicated by + or -, respectively. Lower, mammalian two-hybrid analysis of ADAMTS-7 and TSP-1 interaction. Eahy 926 cells were co-transfected with pACT plasmids that expressed various ADAMTS-7 deletion mutants and pBIND plasmid that encoded



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full-length TSP-1. Luciferase activity was analyzed 48 h after transfection. Data represent the means ± SEM of 3 independent experiments in duplicate. (E) Co-IP of Eahy 926 cells with anti-Flag antibody *in vivo.* Eahy 926 cells were co-transfected with Flag-CMV vectors that encoded various ADAMTS-7 fragments and full length TSP-1 for 48 hours. TSP-1 protein expression was examined by Western blot analysis.

**Figure 6.** TSP1 is cleaved by ADAMTS-7 *in vitro.* Western blot of TSP-1 expression in the cell lysate (A) and conditional medium (B) of HUVECs infected with Ad-*LacZ* and Ad-*ADAMTS-7* respectively. Results are means±SEM from at 4 independent experiments performed. expo. = exposure. (C and D) Western blots analysis of TSP-1. COS-7 cells were infected with Ad-*ADAMTS-7* or Ad-*LacZ* for 24 h. Supernatant was concentrated and incubated with hTSP-1 protein for 4 h *in vitro*. FL TSP-1 = full length TSP-1. Data represent the means ± SEM of 3 independent experiments. (E) Representative western blot of TSP-1. Digestion of hTSP1 by COS-7 cells infected with Ad-*ADAMTS-7* was abolished by an ADAMTS-7 neutralization antibody (50 μg/ml). n=3. (F) Catalytic domain of ADAMTS-7 (TS7-CD) digested hTSP-1. Purified hTSP-1 was incubated with the catalytic domain of ADAMTS-7 *in vitro*. Cleaved products were visualized with Coomassie dye.

**Figure 7.** ADAMTS-7 inhibits endothelial cell recovery through degradation of Thrombospondin-1. (A) Representative Western blots of $siRNA_{scramble}$ and $siRNA_{TSP\text{-}1}$ knockdown of Thrombospondin-1 protein in HUVECs. (B and C) Silencing of TSP-1 attenuated ADAMTS-7–suppressed HUVEC proliferation. HUVECs were transfected with siRNA 24 hours prior to adenoviral infection. Twenty-four hours later, the cells were harvested for CCK-8 or



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FACS analysis (Results are means±SEM from 3 independent experiments performed in duplicate, *$P$<0.05). (D) Knockdown of TSP-1 accelerated Ad-*ADAMTS-7* infected HUVEC migration activity in the wound scratch assay (magnification ×100, Results are means±SEM from 3 independent experiments performed in duplicate, *$P$<0.05). (E) Representative Evans blue staining of *en face* carotid arteries infected by Ad-*LacZ* or Ad-*ADAMTS-7* 5 days after wire injury in *Tsp1*$^{-/-}$ or wild-type mice (Scale bar, 1 mm, re-endothelialization (%) was quantified by Image J, *$P$<0.05; n=6 per group).

**Figure 8.** Schematic illustration of ADAMTS-7 on post-injury neointima formation. On one hand, ADAMTS-7 promotes VSMC migration via degradation of COMP, which is pivotal for VSMC homeostasis. On the other hand, ADAMTS-7 degradation of TSP-1 leads to the generation of bioactive TSP-1-fragments that may mediate retarded re-endothelialization and promote neointima formation.



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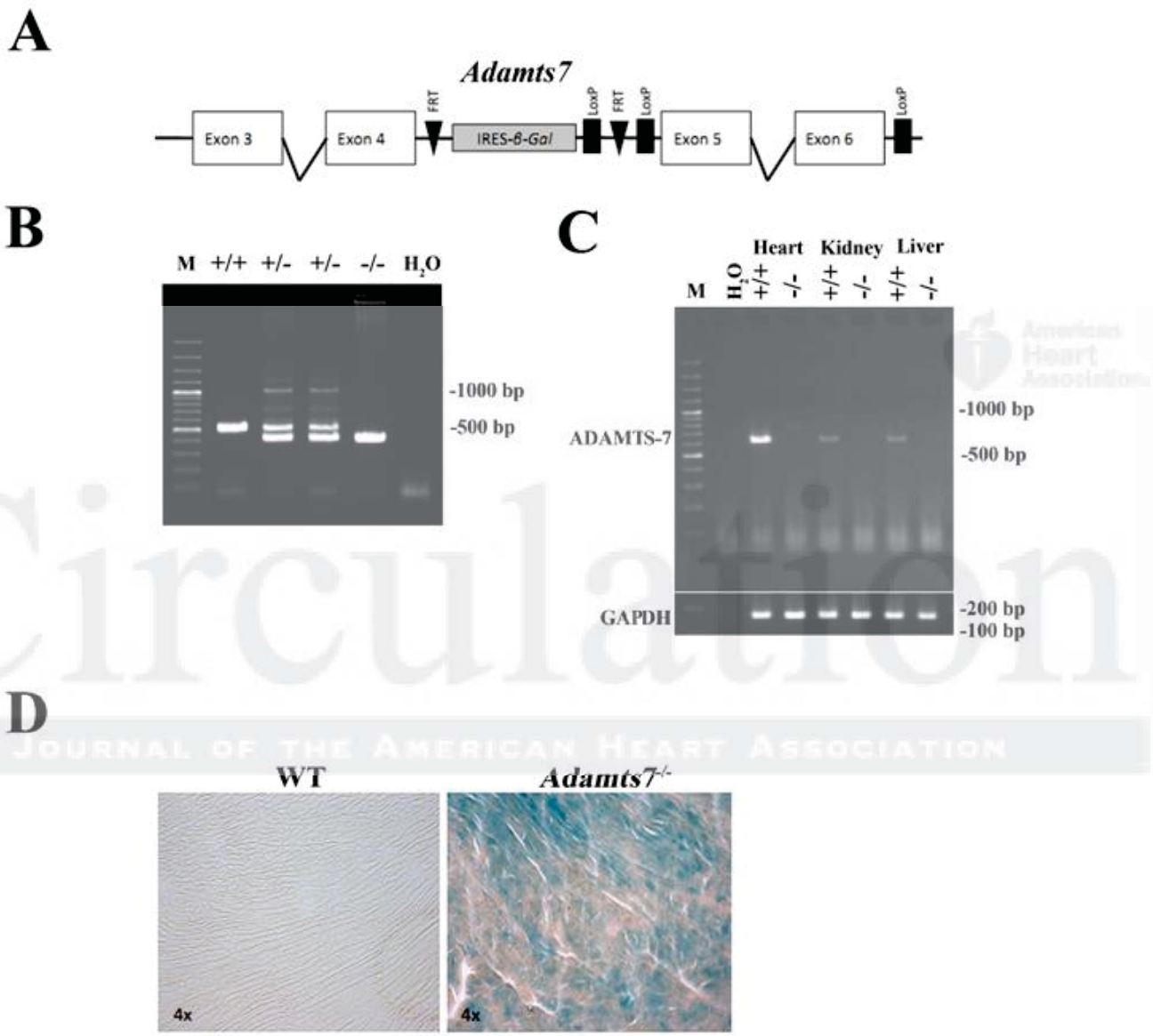


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Figure 1

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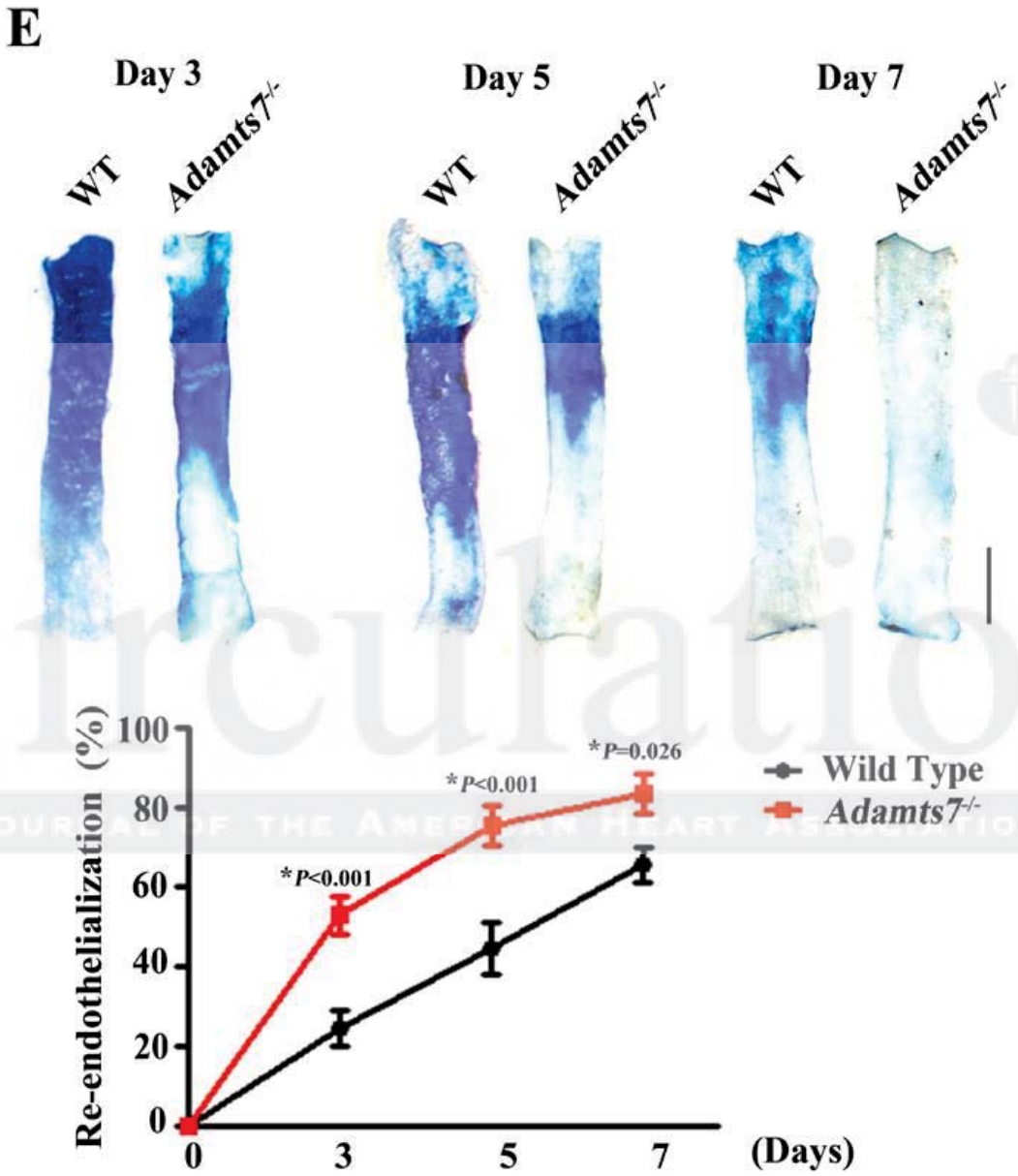


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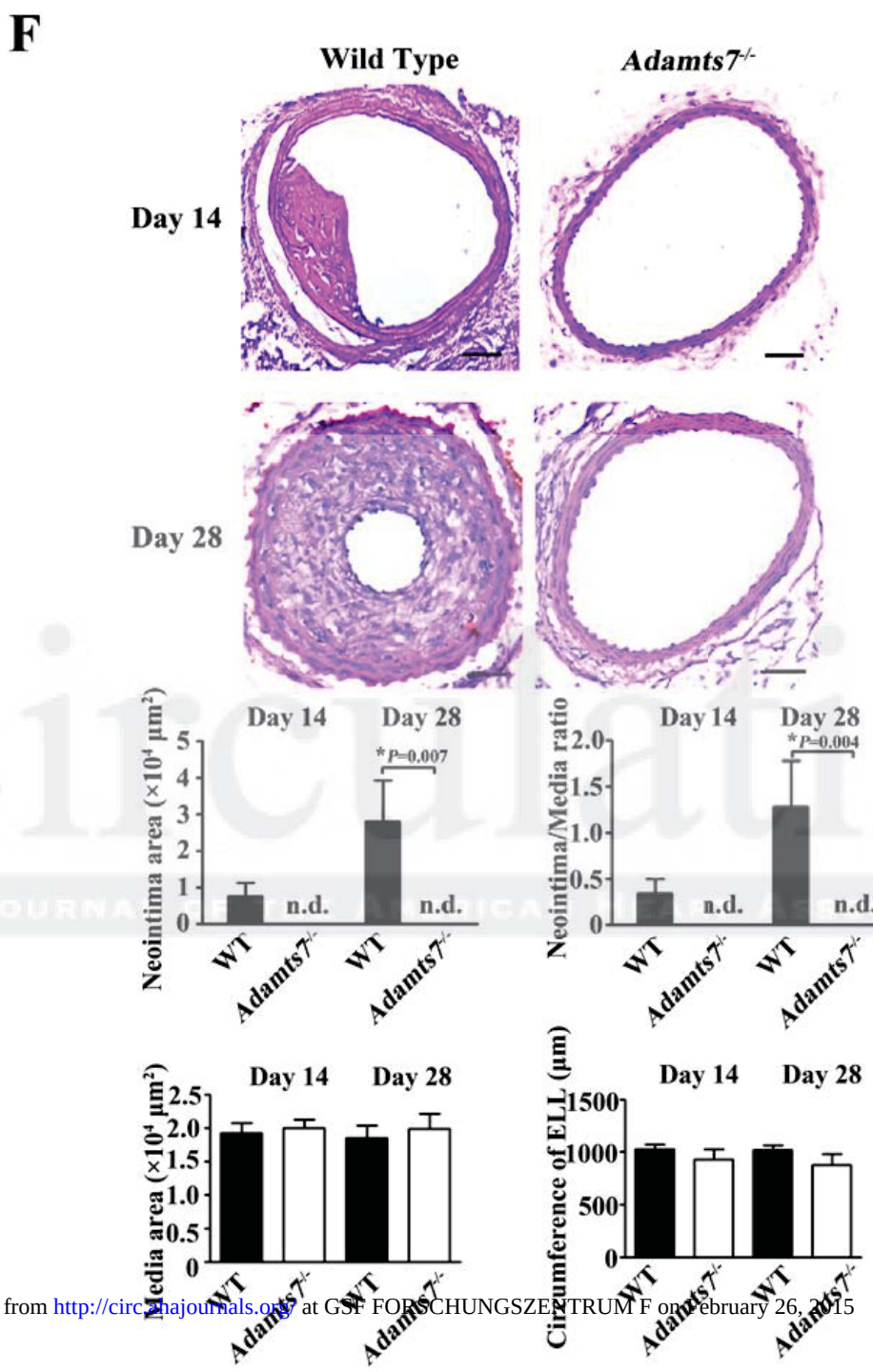


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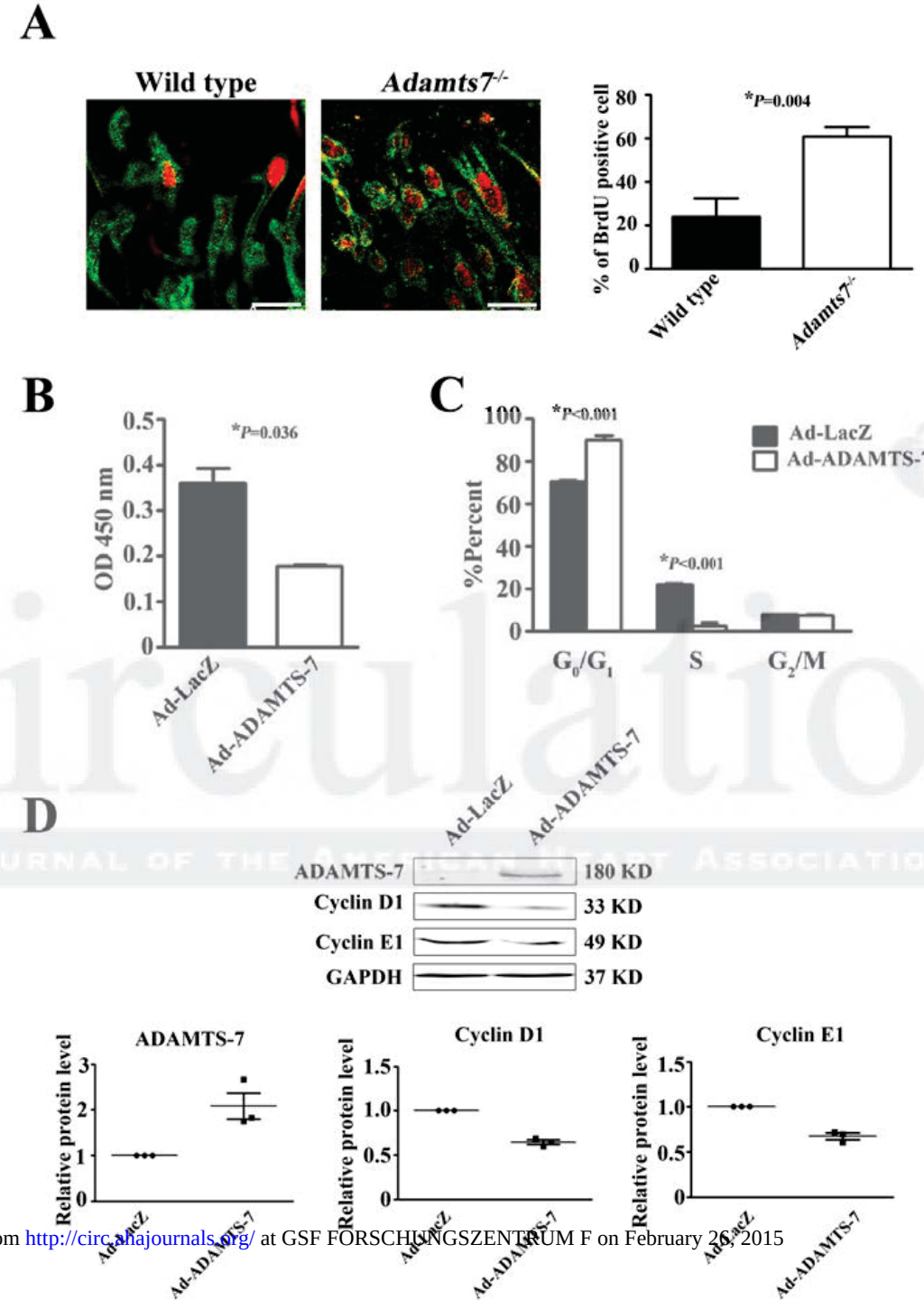


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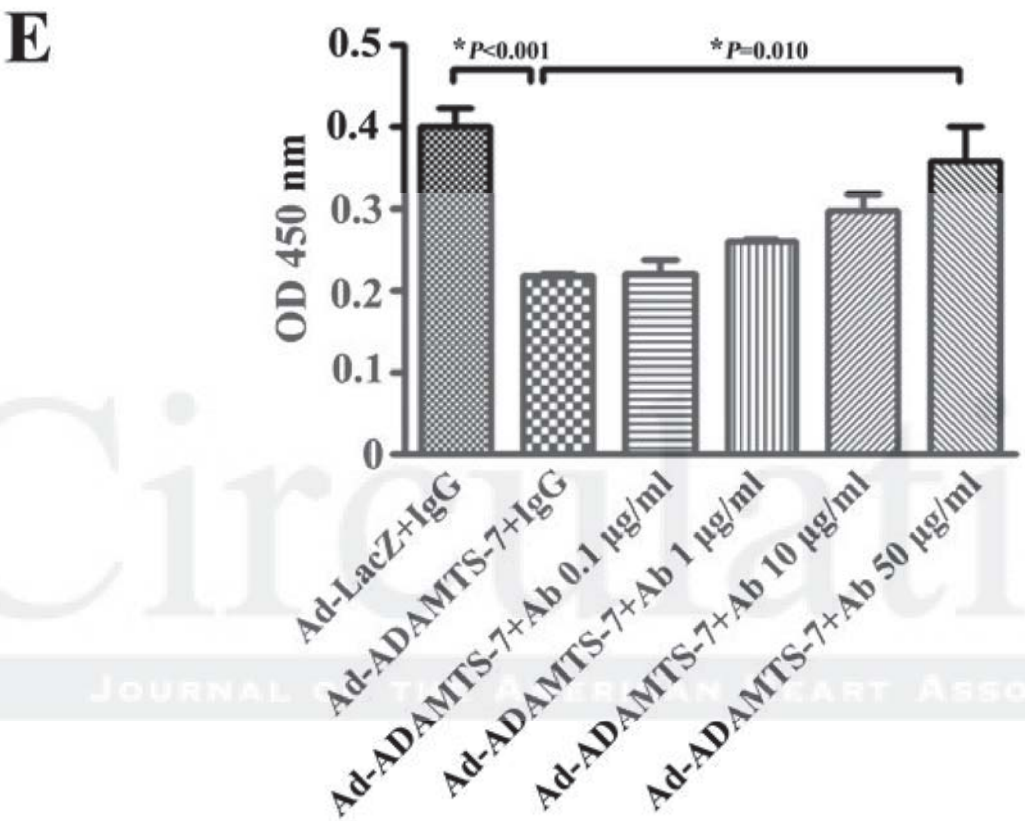


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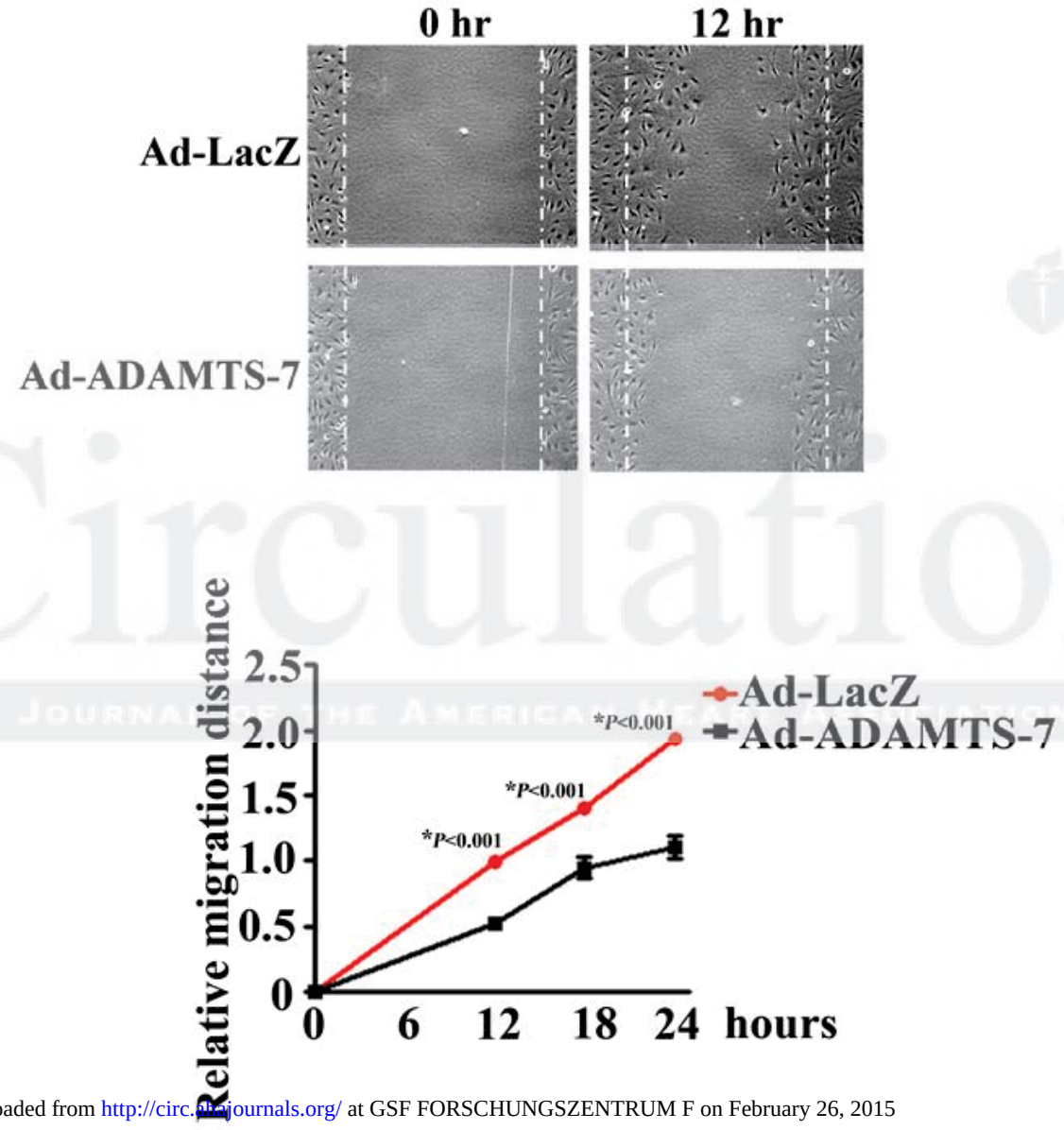


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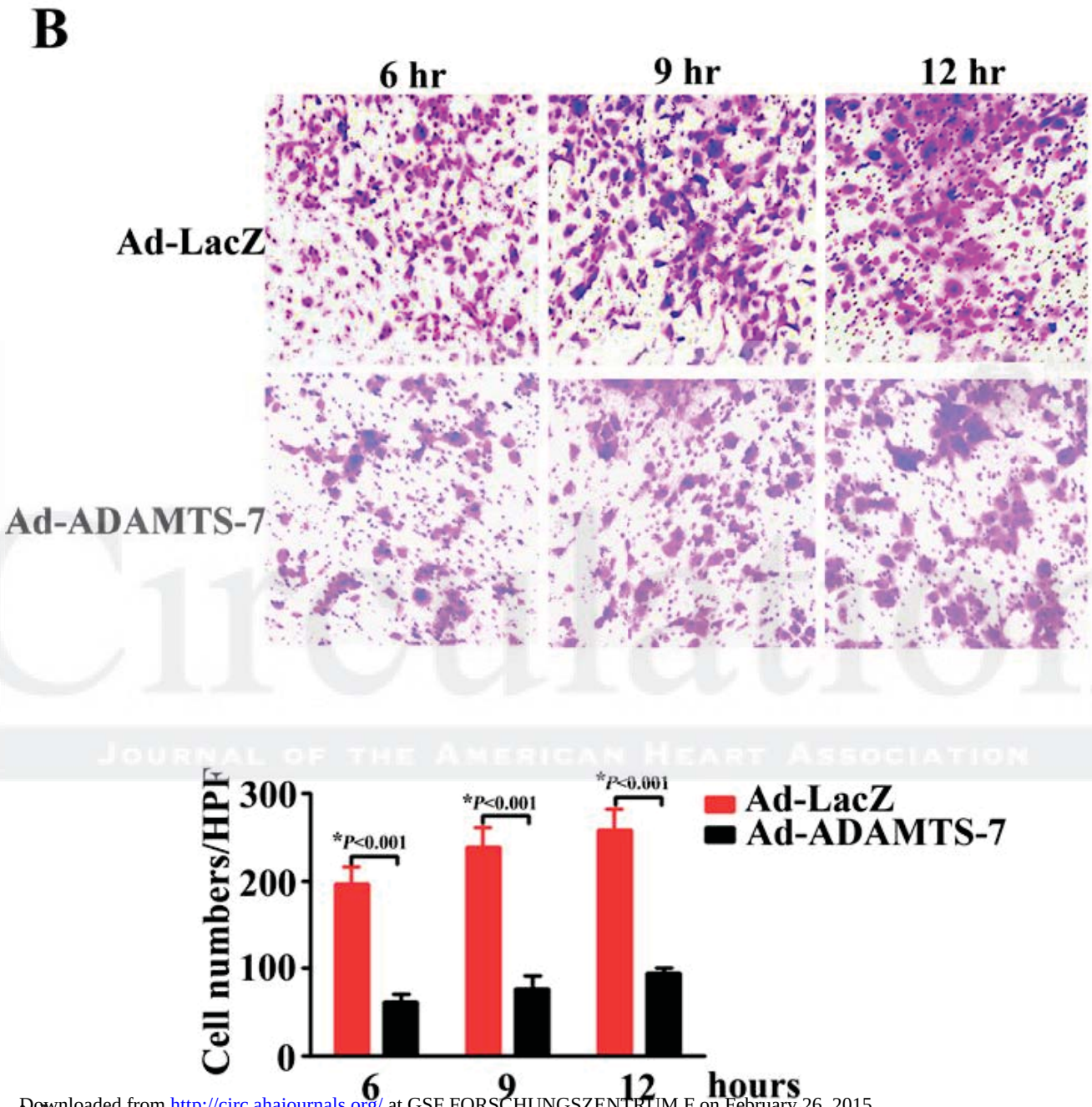


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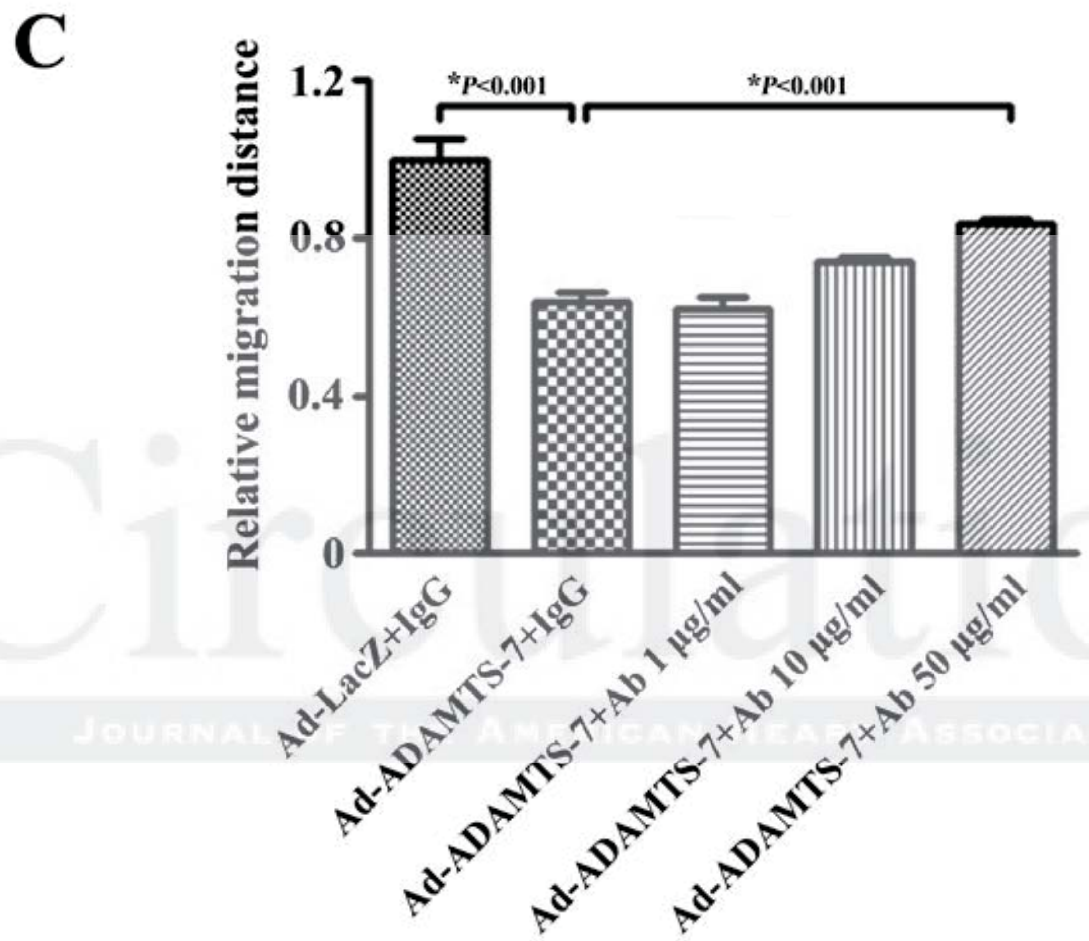


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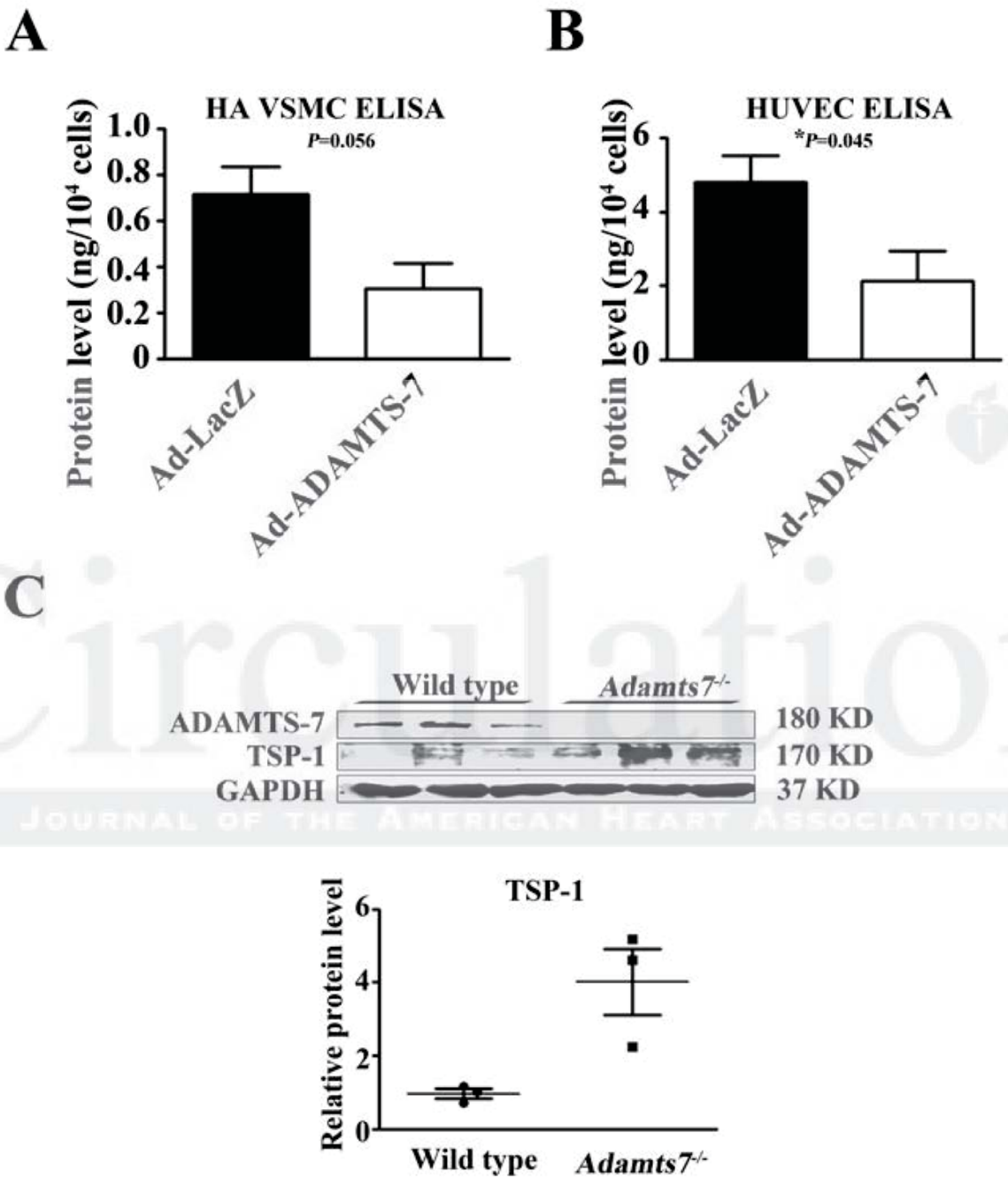


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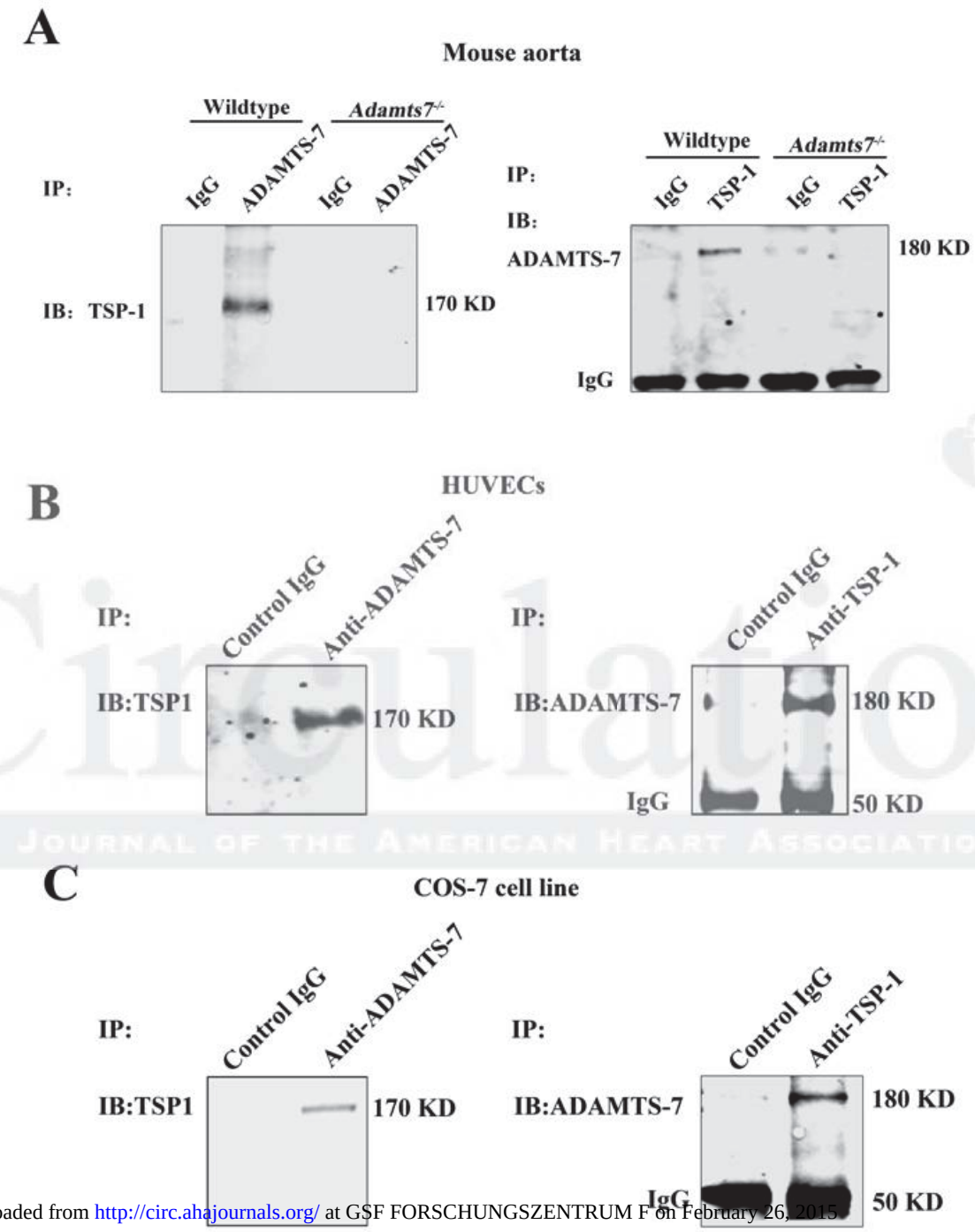


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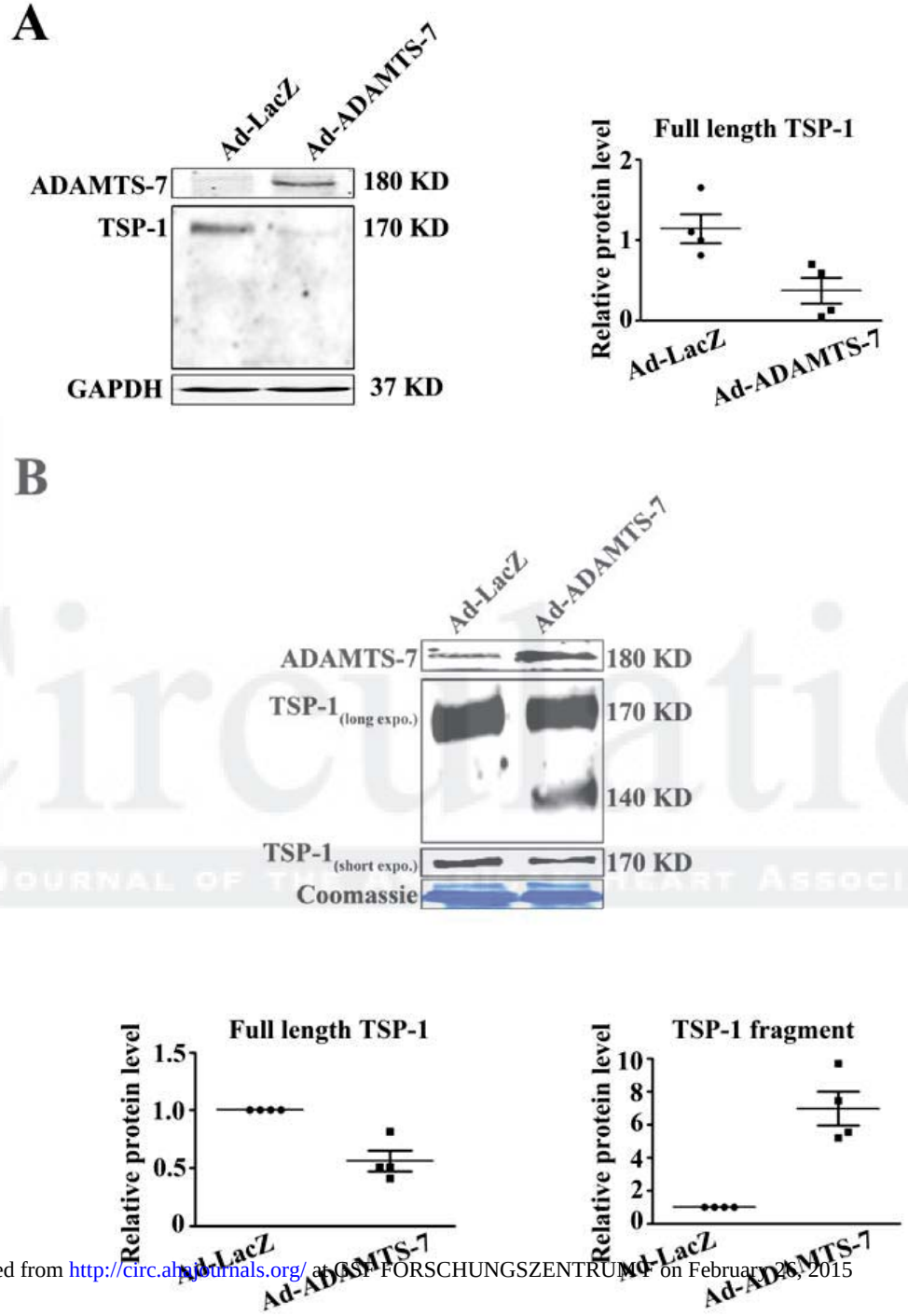


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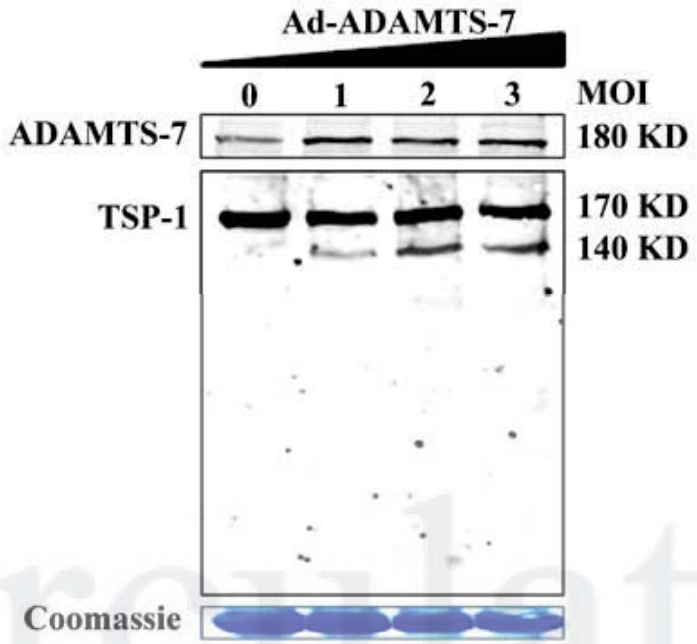


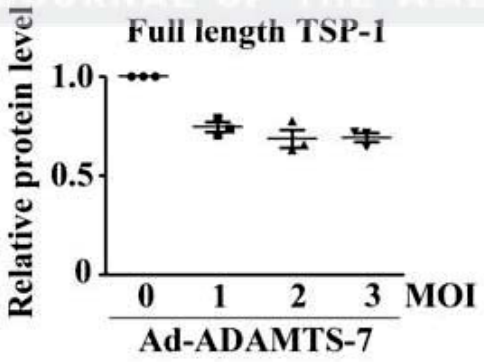


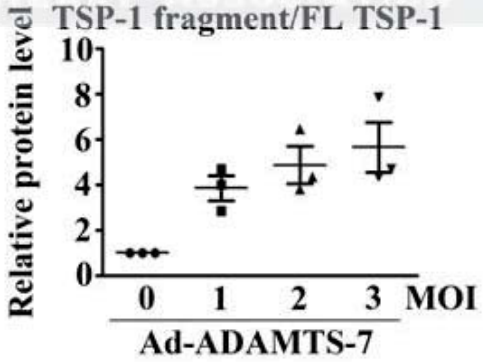


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Figure 6, cont'd

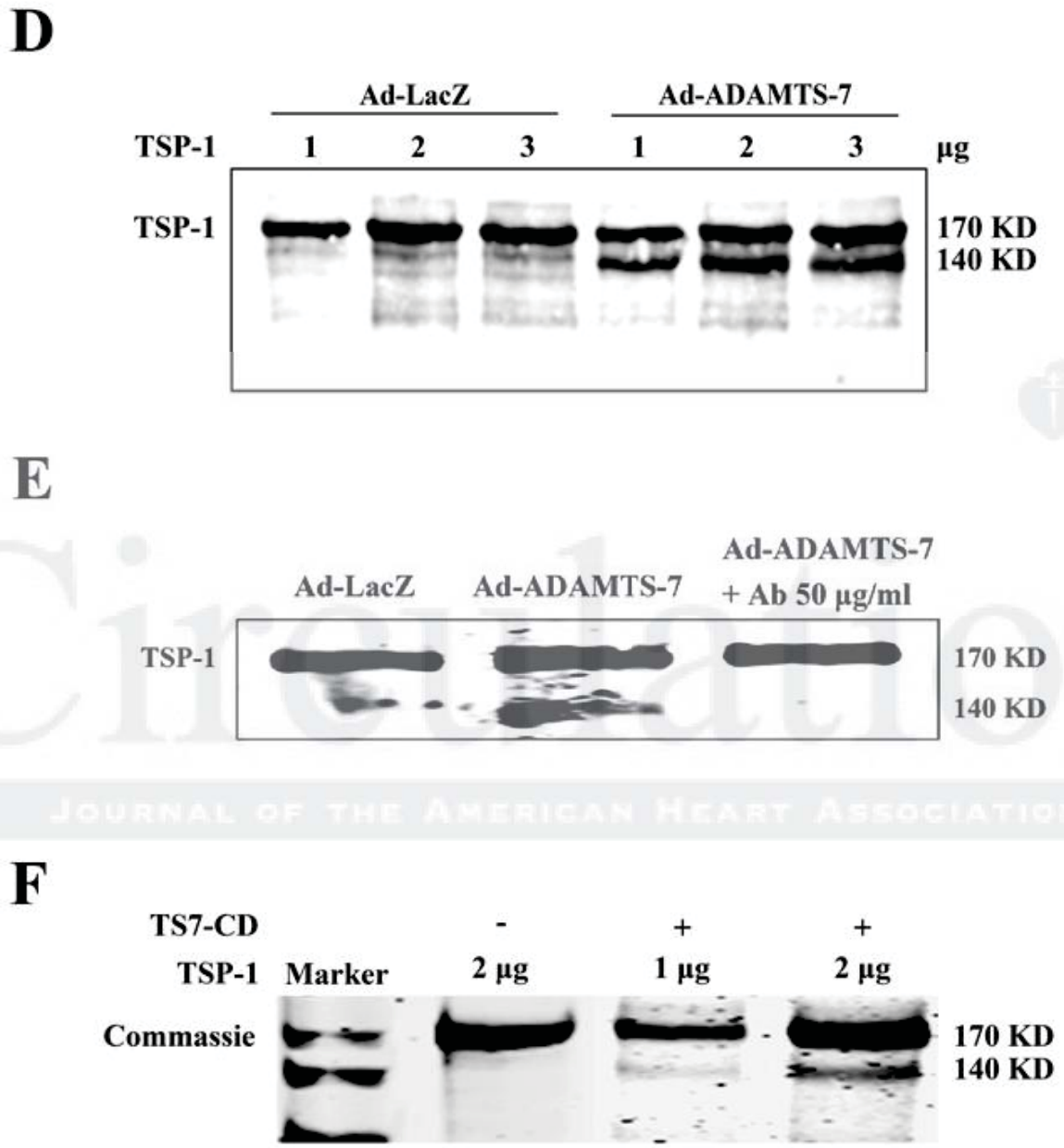


Figure 6, cont'd

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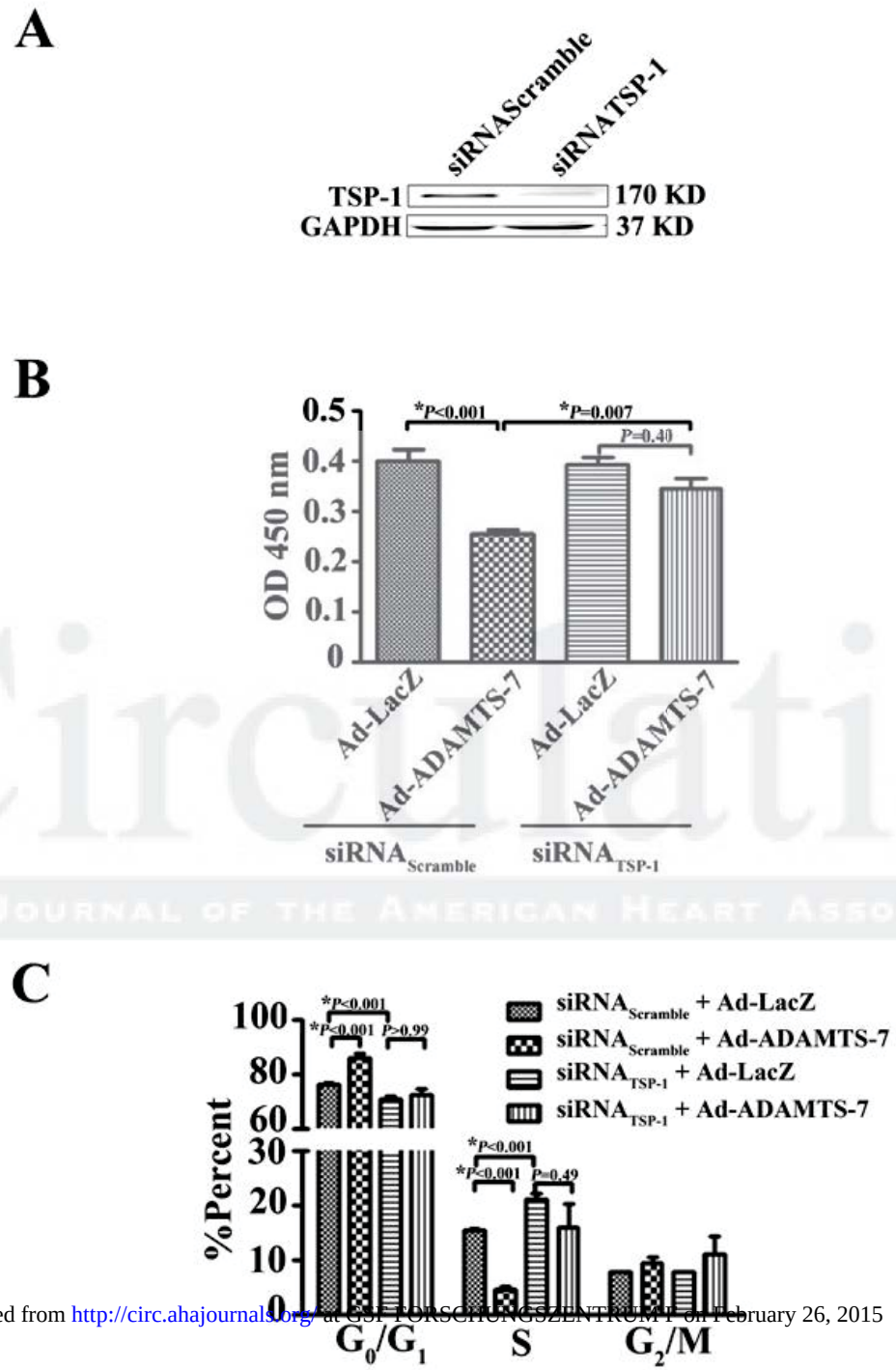


Figure 7

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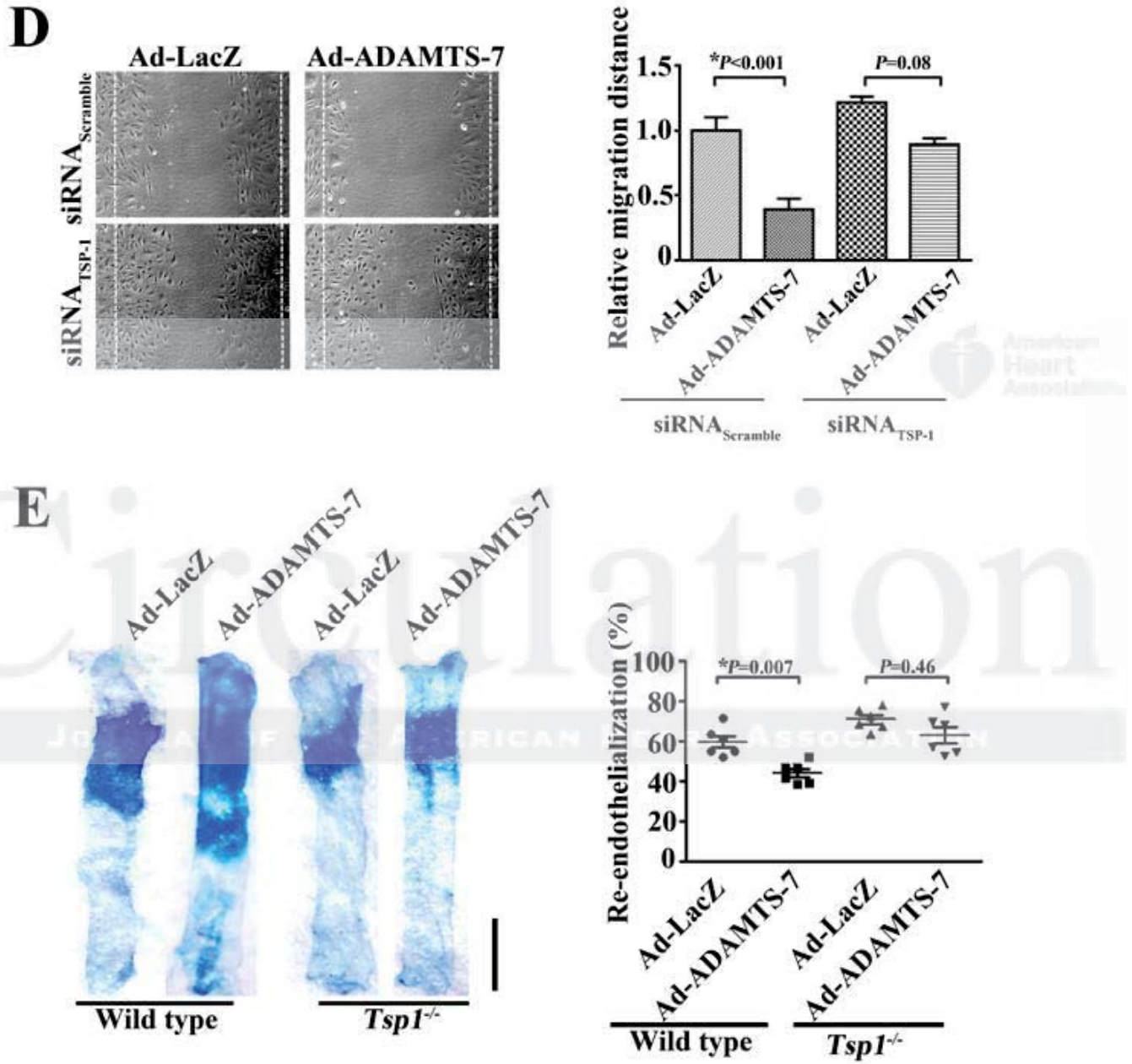


Figure 7, cont'd

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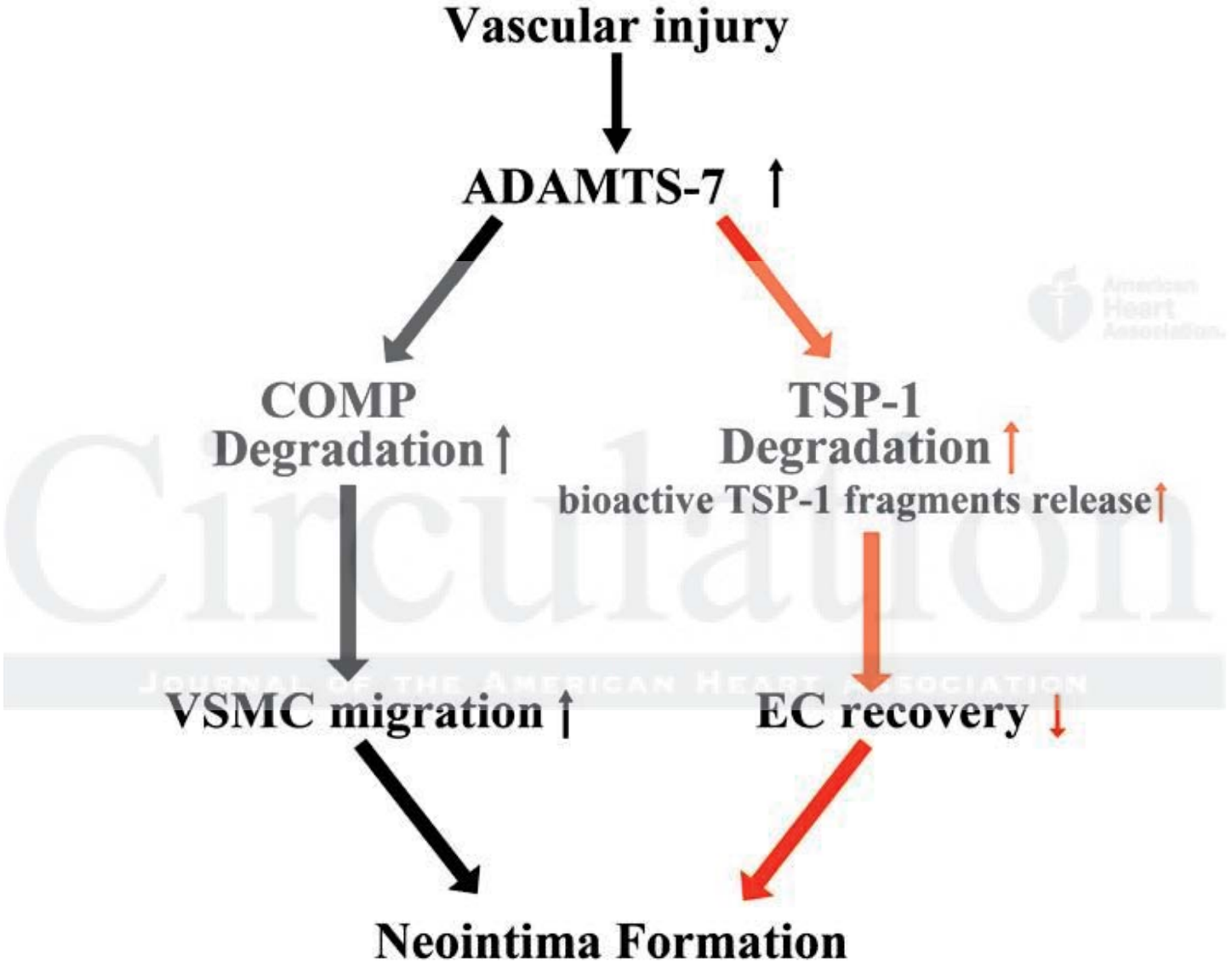


Figure 8

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# SUPPLEMENTAL MATERIAL.

## Supplemental Methods

### Materials

Antibody against ADAMTS-7 was purchased from Abcam (Cambridge, UK). Antibodies against Thrombospondin-1 (TSP-1) were purchased from Neomarkers (Fremont, CA). Antibodies against BrdU were purchased from Sigma-Aldrich (St. Louis, MO). The antibody against GAPDH was purchased from Cell Signaling Technology (Boston, MA). The antibody against vWF was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The antibodies against Cyclin D1 and E1 were purchased from Bioworld (Minneapolis, MN). The neutralizing antibody of ADAMTS-7 (Antigen: LEDEEKDLKITH-KLH) was purchased from Biosynthesis Biotechnology Co., Ltd. (Beijing, China). Recombinant human TSP-1 was purchased from R&D Systems (Minneapolis, MN).

### Generation of *Adamts7*$^{-/-}$ mice

The *Adamts7* knockout embryonic stem cell (*Adamts7*-KO-ESC) line (EPD0209) was purchased from the European Conditional Mouse Mutagenesis Program (EUCOMM)[1]. Microinjection of *Adamts7*-KO-ESC into C57BL/6N blastocysts was achieved at the Knock Out Mouse Project (KOMP) repository at the University of California, Davis, USA[2]. The *Adamts7* targeting vector includes the insertion of a neomycin selection cassette and two loxP sites flanking exon 5 and exon 6 in the *Adamts7* genomic sequence. The *Adamts7* gene was interrupted by introducing an internal ribosome entry site followed by the beta-galactosidase sequence between exons 4 and 5. Male chimeras were obtained and backcrossed to C57BL/6

females in our animal facility to generate founders, which were then selected and genotyped using polymerase chain reaction (PCR) on genomic DNA isolated from ear punch biopsies. Heterozygous mice were intercrossed to generate knockout (*Adamts7*$^{-/-}$), heterozygous (*Adamts7*$^{+/-}$) and wildtype (WT) littermates. Genomic DNA was isolated from ear punches using standard methods. Tissue sections from mice were explanted, snap-frozen in liquid nitrogen and stored at -80°C until use. Homogenization and RNA-isolation were performed using TRIzol (Life Technologies) according to the manufacturer's recommendations. RNA was stored at -80°C until use. cDNA was generated using M-MLV Reverse Transcriptase (Life Technologies) and pdN6-Primers. Amplification of DNA and cDNA was performed using rTaq (GE Healthcare) with the recommended supplements. PCR-products were visualized on agarose-gels.

**X-gal staining of cryosections**

Organs were excised and embedded in Tissue Tek (Sakura), snap frozen in liquid nitrogen, and stored at -20°C until use. The tissues were sectioned into 8-10 µm cryosections. For X-gal staining, the cryosections were air dried, incubated in PBS that contained 0.5% glutaraldehyde at 4°C for 10 min, washed in PBS and incubated with X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) solution at 37°C overnight. Afterwards, the cryosections were washed in PBS, dehydrated and mounted using cover slips (Fisher).

**Large-Scale phenotyping of *Adamts7*$^{-/-}$ mice**

All animal studies followed the guidelines of the Animal Care and Use Committees of

Peking University, People's Republic of China, Schleswig-Holstein and Bavaria, Germany. Fifty-four mice, i.e., 29 females (15 WT, 14 *Adamts7*$^{-/-}$) and 25 males (10 WT, 15 *Adamts7*$^{-/-}$), were generated by intercrossing heterozygous *Adamts7*$^{+/-}$-mice and transferred to the German Mouse Clinic (GMC). At the GMC mice were maintained in IVC cages (Ventirack, Biozone, UK) with water and standard mouse chow (Altromin no. 1324) according to the GMC housing conditions and German laws.The mice were processed by standardized screening procedures as described previously[3, 4]. Briefly, the mice were characterized regarding morphology, behavior, neurology, eye morphology and function, nociception, energy metabolism, clinical chemistry/ hematology, immunology, allergies, steroid metabolism, cardiovascular function, lung function, and pathology.

**Wire-injury of mouse carotid artery**

Wire-injury of the mouse carotid artery was performed in 12-week-old mice as described by Lindner et al[5]. Through a middle line neck incision on the ventral side, the left common carotid artery (LCCA), including bifurcation, was exposed and cleaned from the surrounding tissue. Bulldog clamps were placed around the LCCA proximal to the aortic arch and the left internal carotid artery (LICA) for temporary control of blood flow; a 6-0 suture was placed around the left external carotid artery (LECA). An incision hole was made in the LECA, then a flexible wire (0.38 mm) was introduced into the LCCA by approximately 5 mm and passed 3 times toward and forth with rotation. The wire was removed, and the LECA was then tied off proximally. The skin incision was closed with surgical sutures. The area of remaining denudation at 3, 5 and 7 days after injury was determined by left ventricle injection of Evans

blue dye; quantification of the dye-stained area by blinded image analysis was performed as described previously[6]. Fourteen and 28 days after injury, the mouse arteries were harvested and embedded in Tissue Tek OCT (Sakura Finetek, Staufen, Germany); 6 μm serial cryostat sections were obtained from the bifurcation point and analyzed by matoxylin/eosin staining and Spot Image software (Diagnostic Instruments, Australia). All surgical studies followed the guidelines of the Animal Care and Use Committee of Peking University.

**BrdU incorporation**

BrdU incorporation was performed 3 days after injury as described previously[7]. Briefly, BrdU was administered to mice via an intraperitoneal injection at 48, 24, and 2 hours prior to sacrifice. The carotid arteries were then harvested and incubated with anti-BrdU and anti-vWF antibodies to perform dual immunofluorescence staining.

**Femoral artery injury in mice**

Thrombospondin-1 deficient (*Tsp1*$^{-/-}$) mice were purchased from Jackson Lab. A wire-mediated vascular injury was induced in the femoral arteries of *Tsp1*$^{-/-}$ or WT mice by an angioplasty guide wire as described previously[8]. Briefly, the femoral arteries were exposed by a longitudinal groin incision and monitored under a surgical microscope. The femoral artery was temporarily clamped at the level of the inguinal ligament, and an incision hole was created distal to the deep femoral branch. After release of the clamp, a 0.38-mm guide wire was advanced once by approximately 5 mm and was left in place for 1 minute to denude and dilate the artery. The wire was then removed, and the arteriotomy site was ligated with an 8-0

suture. For *in vivo* overexpression, a single exposure of $5\times10^8$ plaque forming units (pfus) of Ad-ADAMTS7 or Ad-LacZ adenovirus were delivered to the wire-injured femoral artery segments. The skin incision was closed with a 6-0 silk suture. The animals were monitored as per usual after surgery. ADAMTS-7 overexpression *in vivo* was confirmed by immunohistochemistry 3 days after adenovirus delivery (data not shown). Re-endothelialization of the femoral artery was determined by Evans blue staining 5 days after wire injury in mice.

**Immunohistochemistry and Dual Immunofluorescence**

To confirm ADAMTS-7 overexpression in femoral arteries, frozen sections of carotid arteries were incubated with rabbit primary anti-ADAMTS-7 antibody (Abcam, Cambridge, UK), horseradish peroxidase-conjugated goat anti-rabbit IgG and 3, 3-diaminobenzidine, successively. The sections were then counterstained with hematoxylin. For dual immunofluorescence, the frozen sections were first incubated with the antibodies mouse anti-BrdU (1:200) and rabbit anti-vWF (1:50) and then the secondary TRITC-conjugated goat anti-rabbit IgG (1:300) and FITC-conjugated goat anti-mouse IgG (1:300) (Rockland Inc. Gilbertsville, PA), respectively. Fluorescence was detected by confocal laser scanning microscopy (Leica, Germany).

**Cell culture**

HUVECs were isolated from human umbilical veins by type I collagenase (100 IU/ml) and by the differential attachment rate from other cells[9]. Human umbilical cords were

obtained from Peking University Third Hospital. The experiment was approved by the Ethics Committee of the Peking University Health Science Center, and it was conducted after informed consent was provided by the infants' parents. Cells were cultured in medium 199 (Gibco) that contained 10% fetal bovine serum (FBS, Hyclone), 4.17 mg/L recombinant human endothelial cell growth factor (Sigma), 1.4 IU/ml heparin sodium (Sigma), 3.0 mg/L thymidine (Sigma), 5.96 g/L HEPES, 2.2 g/L $NaHCO_3$, 200 U/mL penicillin, and 100 U /mL streptomycin and passaged by 0.05% trypsin digestion. HUVECs of passage 5–6 were used for experiments.

**Real-Time Quantitative PCR and Western Blot Analysis**

Real-time PCR amplification involved the use of an Mx3000 Multiplex Quantitative PCR System (Stratagene Corp, La Jolla, CA) and SYBR Green I reagent normalized to that of the internal control β-actin. The specific primers for human ADAMTS-7 were sense, 5’-GTGGAGACCCTGGTAGTAGC-3’, and antisense, 5’-TCTGCGTGGTGCGTGATCTTTA-3’. The primers for human Thrombospondin-1 were sense, 5’-GACTCCTAGAACGTGCGACCT-3’, and antisense, 5’-CATACAATCGTCTCGGGTATGC-3’, and the primers for human β -actin were sense, 5’-ATCTGGCACCACACCTTC-3’, and antisense, 5’-AGCCAGGTCCAGACGCA-3’. All amplification reactions were conducted over 40 cycles (an initial stage of 7 min at 94°C, then a three-step program of 30 s at 94°C, 30 s at 58°C and 30 s at 72°C) and were performed in duplicate.

Extracts that contained equal amounts of total protein were resolved by 10% or 6-20%

gradient SDS-PAGE. The membranes were incubated with primary antibody and IRDye 700DX-conjugated secondary antibodies (Rockland Inc., Gilbertsville, Pa). The immunofluorescence signal was detected by the Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NE).

**Cell proliferation assay**

After infected with Ad-LacZ or ADAMTS-7 for 48h, HUVECs were trypsinized to single-cell suspension, and 3000 cells in M199 that contained 10% FBS were transferred to each well of a 96-well plate. Cell Counting Kit-8(CCK-8) reagent was added 24 hours after synchronization and incubated at 37°C for 2 to 4 hours according to the color change. The OD (optical density) value at 450 nm was read by a microplate reader (Varioskan Flash, Thermo Fisher). For cell cycle analyses, HUVECs were fixed with 70% ethanol and then stained with 20 μg/mL propidium iodide (PI) and 500 mg/mL RNase A (Sigma), followed by FACS analysis. Each experiment was performed a minimum of 3 times independently.

**Cell migration assay**

HUVECs were infected with Ad-LacZ or ADAMTS-7 for 48h before sratching assay and the modified Boyden Chamber analysis. For the scratching assay, HUVECs ($3 \times 10^5$ cells) were seeded in 6-well plates. The medium was changed to serum-free OPTI-MEM for synchronization after adenoviral infection. Six hours later, scratching was made, and fresh medium that contained 10% FBS was added. Four fields were randomly selected in each well

to record gap distances immediately following scratching at 12, 18 and 24 hours to calculate cell migration.

A modified Boyden Chamber (Chemicon International, MA) coated with an 8-μm barrier of collagen type I was used to test the HUVECs migration ability. For this, 200 μl of suspended HUVECs ($2 \times 10^5$/ml in M199 that contained 10% FBS) were placed in the upper chamber. The lower chamber contained PDGF-BB (20 ng/ml) as a chemoattractant. After 6, 9 or 12 hours, cells on the upper surface were removed by gentle abrasion with the use of a cotton bud, and cells on the underside (invaded cells) were fixed and stained with crystal violet. The mean number of cells on the lower surface was counted from 4 randomly chosen high-power fields (×100) under light microscopy in 3 independent experiments.

**Gel-LC-MS analysis of secretome**

Analysis of the secretome was performed as described[10]. Rat VSMCs were infected with adenovirus that contained GFP or ADAMTS-7. Conditioned media were precipitated with acetone and denatured with 2× SDS sample buffer (Invitrogen) at 97°C for 5 min. Proteins were separated on 4%-12% NuPAGE Bis-Tris gels (Invitrogen). After silver staining, each lane was cut into 16 gel bands without gaps and digested with trypsin (Promega) using a robotic digestor (ProGest, Digilab) overnight. Peptides were separated by nano-flow HPLC on a reverse-phase column (C18 PepMap 100, 3 μm, 100 Å, 25 cm; Thermo Fisher Scientific) and identified by a LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific). Spectra were acquired with the full MS scan range of m/z 450-2000 followed by six dependent MS2

scans using dynamic exclusion. The results were blasted against the UniProt/SwissProt database (SwissProt 57.15, 515203 entries) using Mascot server 2.3.01 (Matrix Science). The following parameters were used: peptide tolerance = 10 ppm, fragment tolerance = 0.8 Da, carbamidomethylation of cysteine as fixed modification, oxidation of methionine as variable modification, and 2 missed cleavages were allowed. Scaffold (version 3.6.2, Proteome Software) was used to validate MS/MS-based peptide and protein identification with the following filters: peptide probability > 95%, protein probability > 99%, and minimum no. peptides per protein ≥ 2.

**Co-Immunoprecipitation**

Cells or rat aorta artery lysates were incubated with anti-ADAMTS-7 or anti-TSP-1 antibodies prior to immunoprecipitation with protein P/A agarose beads (Santa Cruz, CA). The precipitated proteins were resolved by 10% SDS-PAGE and immunoblotted with anti-TSP-1 or anti-ADAMTS-7 antibodies, respectively. Rabbit or mouse IgG antibodies served as a negative control.

In another site of study, Eahy 926 cells were co-transfected with Flag-CMV vectors that encoded various ADAMTS-7 fragments (Flag-TS7(26-246), Flag-TS7(238-711), Flag-TS7(703-1007), and Flag-TS7(999-1595) and full length TSP-1 for 48 hours respectively. Cell lysates were incubated with anti-Flag antibody and then immunoblotted with anti-TSP-1 antibodies.

**TSP1 siRNA Transfection**

Small interfering RNA (siRNA) against human TSP-1 was purchased from GenePharma Co., Ltd (Shanghai). Sequences corresponding to the siRNA of TSP-1 were sense, 5'-GCGUGUUUGACAUCUUUGATT-3', and antisense, 5'-UCAAAGAUGUCAAACACGCTT-3'. Transfection of HUVECs with siRNA (20 nmol/L) *in vitro* was performed using RNAi MAX (Invitrogen). A scramble stealth RNAi duplex served as a negative control.

**Subcloning TSP-1 plasmid**

The cDNA fragment encoding the full-length human *TSP1* (NCBI Reference sequence: NM_003246.2) was cloned into the *SalI/XbaI* sites of pDNR-CMV and pFlag-CMV plasmid. The primer sequences were sense, 5'-TGCTCTAGAACAGGATCCCTGCTGGGCACCAACA-3', and antisense, 5'-CGGGGTACCTCCAGAAGGTGCAATACCAGCATTGG-3'.

**Mammalian two-hybrid assay**

The fragments that encoded the 4 functional domains of rat *ADAMTS-7* (i.e., the prodomain [TS7(26-246); aa 26-246], the metalloproteinase plus disintegrin-like and cysteine-rich domain [TS7(238-711); aa 238-711], the spacer-1 plus three TSP repeats [TS7(703-1007); aa 703-1007], and the spacer-2 plus four C-terminal TSP repeats [TS7(999-1595); aa 999-1595]) were amplified by PCR and subcloned in-frame into the *Sal*I/*Xba*I or *Ecor*V/*Xba*I sites of pACT (pACT-TS7(26-246), pACT-TS7(238-711), pACT-TS7(703-1007), and pACT-TS7(999-1595), respectively. cDNA inserts that encoded

human TSP-1 were subcloned in-frame into the pBIND vector to generate the indicated plasmids (pBIND-TSP-1). Eahy 926 cells were cotransfected with the target and bait constructs, together with the reporter plasmid pG5luc-luciferase at a ratio of 1:1:1. After 48 h, the transfected cells were harvested, and the cell lysates were used for a luciferase assay with the Dual-Luciferase Reporter Assay System (Promega). The fragment primer sequences are listed in Supplemental Table 6.

**Mutagenesis of TSP-1**

The ADAMTS-1 cleavage site in TSP-1 (glutamic acid 311 and leonine 312)[11] were mutated to Isoleucine and Asparagine respectively. Site-mutation was mediated by DpnI-Restriktionsendonuklease (Takara).

***In vitro* digestion of TSP1**

The digestion assay was performed as described previously[11]. Briefly, COS-7 cells were infected with adenovirus that expressed LacZ (control) or ADAMTS-7 for 48 hours. The medium was then changed to serum-free DMEM. The culture medium (CM) was collected after 24 h of incubation and concentrated by centrifugal filter devices (Amicon Ultra-0.5, Millipore). Purified hTSP-1 (R&D) was incubated with CM from adenoviral infected cells at 37°C for 4 hours.

**Cleavage of TSP-1 by recombinant catalytic domain of ADAMTS-7 *in vitro***

The bacterial expression vector pGEX-6p-1 was used to produce recombinant

glutathione S-transferase (GST) fusion proteins in *Escherichia coli*. The cDNA fragments that encoded a catalytic domain-containing segment of rADAMTS-7 (aa 217-427) were subcloned into the *BamHI/XhoI* site. The primer sequences were sense: 5'-CGCGGATCCTCAATCAGCAAAGAGAAGTG-3', and antisense: 5'-CCGCTCGAGGGACGGTCATCTAAGCACAG-3'. Purified hTSP-1 was incubated with the bacteria-expressed catalytic domain of ADAMTS-7 in a digestion buffer (50 mM Tris-HCl, 100 mM NaCl, 5 mM $CaCl_2$, 2 mM $ZnCl_2$, and 0.05% Brij-35, pH 7.5) at 37°C for 4 h[12].

**Isolation of the mouse aortic ECs and VSMCs.**

After PBS perfusion, the mouse arteries were harvested and dissected longitudinally. Endothelium was carefully scraped in PBS and collected from 7 mice by centrifugation. The precipitate was resuspended with 60 μl lysis buffer. For VSMC isolation, media of the aorta were tore up and grinded in lysis buffer. Expression of TSP-1 was analyzed with Western blot.

**Statistical Analysis**

All results were expressed as the mean ± standard error of the mean (SEM). Statistical analysis involved the use of Mann-Whitney U test for comparison of two groups to evaluate the effects of ADAMTS-7 on the BrdU incorporation, cell proliferation, and TSP-1 concentration by ELISA analysis in cell condition medium, to analyze the role of COMP on HUVEC proliferation, to assess the postinjury neointima area in WT and *comp*$^{-/-}$ mice. Comparisons among more than 2 groups involved non-parametric Kruskal-Wallis test with a

Dunn's post-hoc test to evaluate the effects of ADAMTS-7 neutralization antibody on cell proliferation and migration. Comparison of more than 2 groups involved two-way ANOVA followed by the Bonferroni test for post-hoc comparison as appropriate to evaluate the effects of ADAMTS-7 on re-endothelialization, neointima formation, the cell cycle and cell migration, as well as the effect of TSP-1 on ADAMTS-7 mediated cell proliferation, migration and re-endothelialization. Statistical analyses involved the use of GraphPad Prism 6.0 (GraphPad Software Inc, La Jolla, CA). All *P* -values were two-sided and a $P<0.05$ was considered statistically significant.

**Supplemental Table 1. Large-scale phenotyping of the *Adamts7*$^{-/-}$ mouse (in cooperation with the German Mouse Clinic).**

| Screens | Phenotype of *Adamts7*$^{-/-}$-mice |
|---|---|
| Behaviour | Decreased anxiety in open field test |
| Neurology | Reduced rotarod latency in females |
| Nociception | None |
| Dysmorphology | None |
| *Clinical Chemistry* | *Mild effects on triglycerides and red blood cell count* |
| *Energy Metabolism* | *Increased maximum oxygen consumption in males* |
| *Cardiovascular* | *None* |
| Eye | None |
| Immunology | None |
| Allergy | None |
| Steroid Metabolism | None |
| Lung function | Increase in lung function parameters, reduced resistance |
| *Pathology* | *None* |

# Supplemental Table 1

**Supplemental Table 2. Phenotyping of *Adamts7*$^{-/-}$ mice (KO) compared to WT mice regarding energy metabolism, clinical chemistry and hematology. Data are mean ± SD.**

| Test | Female | | Male | | Linear model | | |
|---|---|---|---|---|---|---|---|
| | | | | | sex | genotype | sex:genotype |
| **Energy metabolism** | **WT (n=7)** | **KO (n=8)** | **WT (n=7)** | **KO (n=8)** | | | |
| Avg. mass [g] | 21.8 ± 2.2 | 21.3 ± 1.1 | 28.1 ± 1 | 28.3 ± 1.6 | <0.001 | 0.77 | 0.556 |
| Food intake [g] | 1.8 ± 0.7 | 1.4 ± 0.8 | 2.1 ± 0.7 | 2.4 ± 0.5 | 0.571 | 0.996 | 0.176 |
| Max. VO2 [ml/(h animal)] | 115.57 ± 18.14 | 117.88 ± 9.42 | 119.86 ± 7.36 | 134.63 ± 16.14 | 0.002 | 0.014 | 0.261 |
| Avg. distance [cm] | 5928 ± 1270 | 6532 ± 1983 | 4837 ± 3106 | 4576 ± 1624 | 0.057 | 0.824 | 0.577 |
| | | | | | | | |
| **Clinical chemistry** | **WT (n=15)** | **KO (n=14)** | **WT (n=10)** | **KO (n=15)** | | | |
| Fasting glucose [mM] | 5.79 ± 0.75 | 6.3 ± 0.86 | 6.63 ± 0.86 | 7.16 ± 1.37 | 0.002 | 0.056 | 0.965 |
| Creatinine [µM] | 10.43 ± 1.57 | 91.3 ± 2.7 | 9.65 ± 1.37 | 10.99 ± 1.42 | 0.298 | 0.973 | 0.013 |
| Triglycerides [mM] | 0.98 ± 0.28 | 0.86 ± 0.17 | 1.80 ± 0.31 | 1.49 ± 0.26 | <0.001 | 0.003 | 0.177 |
| Cholesterol [mM] | 1.77 ± 0.26 | 1.69 ± 0.21 | 2.03 ± 0.28 | 1.98 ± 0.30 | <0.001 | 0.377 | 0.818 |
| ALP [U/l] | 146 ± 15 | 157 ± 11 | 84 ± 8 | 83 ± 10 | <0.001 | 0.137 | 0.06 |
| | | | | | | | |
| **Hematology** | **WT (n=15)** | **KO (n=14)** | **WT (n=10)** | **KO (n=15)** | | | |
| RBC [$10^6$/mm$^3$] | 10.85 ± 0.24 | 10.58 ± 0.39 | 10.77 ± 0.22 | 10.62 ± 0.36 | 0.844 | 0.02 | 0.503 |
| HGB [g/dl] | 16.51 ± 0.29 | 15.96 ± 0.53 | 16.13 ± 0.39 | 15.95 + 0.48 | 0.105 | 0.004 | 0.139 |
| WBC [$10^3$/mm$^3$] | 7.55 ± 2.04 | 6 ± 0.88 | 7.65 ± 1.5 | 8.79 ± 1.52 | 0.001 | 0.635 | 0.003 |
| PLT [$10^3$/mm$^3$] | 1281.6 ± 106.47 | 1316.79 ± 97.55 | 1331.8 ± 113.71 | 1291.6 ± 201.71 | 0.746 | 0.948 | 0.331 |

**Supplemental Table 3. Phenotyping of *Adamts7*$^{-/-}$ mice (KO) compared to WT mice regarding pulmonary function. Data are mean [CI].**

| Test | Female WT n=5 | Female KO n=6 | p-value |
|---|---|---|---|
| Tidal volume, ml | 0.22 [0.22-0.22] | 0.23 [0.23-0.24] | 0.011 |
| Vital capacity, ml | 0.98 [0.91-1.16] | 1.23 [1.19-1.50] | 0.061 |
| Funct. residual capacity, ml | 0.25 [0.23-0.25] | 0.32 [0.28-0.36] | 0.024 |
| Residual volume, ml | 0.015 [0.008-0.02] | 0.025 [0.015-0.0.033] | 0.4 |
| Total lung capacity, ml | 0.96 [0.89-1.17] | 1.26 [1.16-1.39] | 0.052 |
| Forced vital capacity, ml | 0.89 [0.81-1.08] | 1.24 [1.05-1.43] | 0.19 |
| Forced exspiratory volume, ml | 0.87 [0.79-1.04] | 1.195 [1.035-1.35] | 0.111 |
| Dynamic lung compliance, ml/cm$H_2O$ | 0.02 [0.02-0.02] | 0.03 [0.03-0.03] | 0.015 |
| Lung resistance, cm$H_2O$/ml/s | 1.36 [1.34-1.39] | 1.25 [1.22-1.29] | 0.126 |

**Supplemental Table 4. Blood lipid levels after 15 weeks of western diet comparing WT mice and *Adamts7*$^{-/-}$ mice (KO). Data are mean ± SD.**

| **Test** | **WT**<br>**n=8** | **KO**<br>**n=5** | **p-value** |
|---|---|---|---|
| Cholesterol, mmol/l | 5.17 ± 1.81 | 4.83 ± 1.16 | 0.721 |
| LDL-cholesterol, mmol/l | 0.53 ± 0.19 | 0.52 ± 0.09 | 0.916 |
| HDL-cholesterol, mmol/l | 2.10 ± 0.53 | 2.06 ± 0.41 | 0.879 |
| Triglycerides, mmol/l | 0.58 ± 0.12 | 0.60 ± 0.15 | 0.790 |

**Supplemental Table 5. Differentially expressed proteins in the conditioned media of Ad-*GFP* (G) and Ad-*ADAMTS-7* (T) SMCs.**

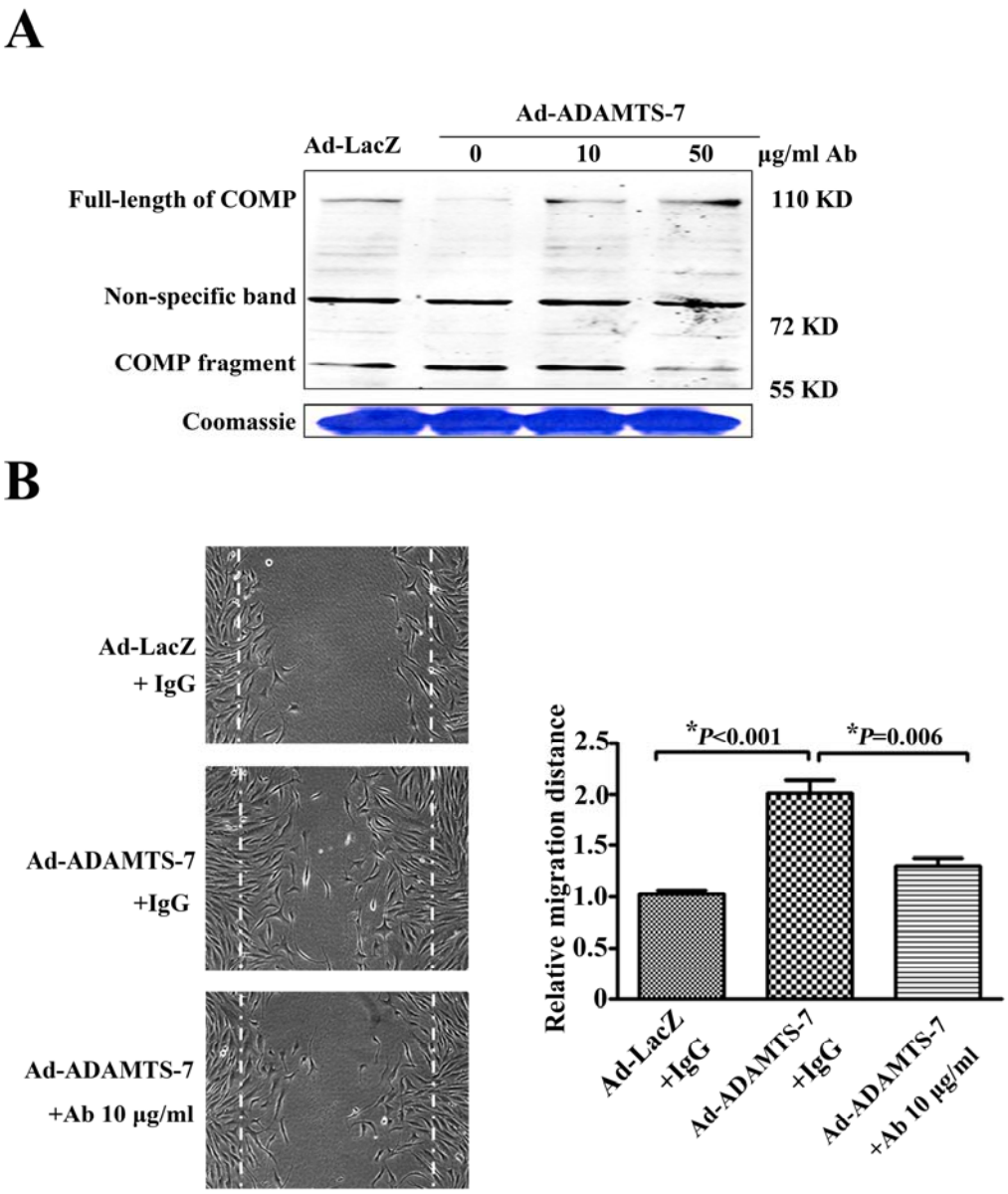
| Protein Name | UniProt ID | Mw | T-Test (*P*-Value) | Fold Change (T/G) | Normalized Spectral Count | | | Normalized Spectral Count | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | G1 | G2 | G3 | T1 | T2 | T3 |
| 6-phosphogluconate dehydrogenase, decarboxylating | 6PGD_RAT | 53 kDa | 0.00003 | Infinite | 0.0 | 0.0 | 0.0 | 5.5 | 6.4 | 6.4 |
| A disintegrin and metalloproteinase with thrombospondin motifs 7 | ATS7_RAT | 176 kDa | 0.00020 | Infinite | 0.0 | 0.0 | 0.0 | 152.9 | 189.0 | 150.2 |
| Actin-related protein 3 | ARP3_RAT | 47 kDa | 0.049 | 2.95 | 0.0 | 5.8 | 2.9 | 10.1 | 9.1 | 6.4 |
| Alpha-actinin-1 | ACTN1_RAT | 103 kDa | 0.016 | 1.41 | 30.5 | 34.9 | 30.7 | 45.1 | 50.2 | 40.1 |
| Alpha-actinin-4 | ACTN4_RAT | 105 kDa | 0.030 | 1.29 | 31.7 | 38.4 | 37.4 | 44.2 | 51.1 | 43.7 |
| Elastin | ELN_RAT | 73 kDa | 0.048 | -1.90 | 79.3 | 78.0 | 51.8 | 51.6 | 32.0 | 26.4 |
| Elongation factor 2 | EF2_RAT | 95 kDa | 0.027 | 2.26 | 4.9 | 4.7 | 3.8 | 12.9 | 10.0 | 7.3 |
| Growth/differentiation factor 6 | GDF6_RAT | 51 kDa | 0.017 | -Infinite | 8.5 | 4.7 | 3.8 | 0.0 | 0.0 | 0.0 |
| Guanine nucleotide-binding protein subunit beta-2-like 1 | GBLP_RAT | 35 kDa | 0.019 | 6.75 | 2.4 | 0.0 | 0.0 | 4.6 | 7.3 | 4.6 |
| Heterogeneous nuclear ribonucleoprotein K | HNRPK_RAT | 51 kDa | 0.0010 | Infinite | 0.0 | 0.0 | 0.0 | 4.6 | 6.4 | 4.6 |
| Heterogeneous nuclear ribonucleoprotein Q | HNRPQ_RAT | 60 kDa | 0.0020 | Infinite | 0.0 | 0.0 | 0.0 | 3.7 | 3.7 | 5.5 |
| Importin subunit beta-1 | IMB1_RAT | 97 kDa | 0.031 | Infinite | 0.0 | 0.0 | 0.0 | 2.8 | 6.4 | 2.7 |
| Low molecular weight phosphotyrosine protein phosphatase | PPAC_RAT | 18 kDa | 0.031 | Infinite | 0.0 | 0.0 | 0.0 | 2.8 | 6.4 | 2.7 |
| Moesin | MOES_MOUSE | 68 kDa | 0.0065 | 2.18 | 8.5 | 7.0 | 6.7 | 13.8 | 19.2 | 15.5 |
| Olfactomedin-like protein 3 | OLFL3_RAT | 46 kDa | 0.046 | -1.56 | 7.3 | 9.3 | 7.7 | 6.4 | 3.7 | 5.5 |
| Osteopontin | OSTP_RAT | 35 kDa | 0.023 | -5.80 | 20.7 | 17.5 | 9.6 | 2.8 | 5.5 | 0.0 |
| Periostin | POSTN_MOUSE | 93 kDa | 0.023 | -1.60 | 34.2 | 32.6 | 32.6 | 21.2 | 14.6 | 26.4 |
| Peroxiredoxin-5, mitochondrial | PRDX5_RAT | 22 kDa | 0.040 | 5.95 | 0.0 | 0.0 | 3.8 | 5.5 | 11.0 | 6.4 |
| Pigment epithelium-derived factor | PEDF_MOUSE | 46 kDa | 0.0066 | -1.49 | 32.9 | 31.4 | 29.7 | 19.3 | 19.2 | 24.6 |
| Proliferation-associated protein 2G4 | PA2G4_MOUSE | 44 kDa | 0.00064 | Infinite | 0.0 | 0.0 | 0.0 | 3.7 | 2.7 | 2.7 |
| Protein DJ-1 | PARK7_RAT | 20 kDa | 0.0057 | Infinite | 0.0 | 0.0 | 0.0 | 4.6 | 2.7 | 2.7 |
| Pyruvate kinase isozymes M1/M2 | KPYM_RAT | 58 kDa | 0.030 | 2.13 | 7.3 | 9.3 | 18.2 | 24.9 | 28.3 | 20.9 |
| Septin-11 | SEP11_RAT | 50 kDa | 0.012 | 7.94 | 0.0 | 0.0 | 2.9 | 6.4 | 6.4 | 10.0 |
| Sushi repeat-containing protein SRPX | SRPX_RAT | 52 kDa | 0.018 | -1.92 | 8.5 | 11.6 | 9.6 | 5.5 | 3.7 | 6.4 |
| Sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1 | SVEP1_RAT | 387 kDa | 0.0027 | -8.51 | 4.9 | 5.8 | 4.8 | 0.0 | 0.0 | 1.8 |
| T-complex protein 1 subunit theta | TCPQ_MOUSE | 60 kDa | 0.0014 | Infinite | 0.0 | 0.0 | 0.0 | 5.5 | 5.5 | 3.6 |
| Thioredoxin | THIO_RAT | 12 kDa | 0.027 | 3.04 | 0.0 | 4.7 | 2.9 | 8.3 | 8.2 | 6.4 |
| Thrombospondin-1 | TSP1_MOUSE | 130 kDa | 0.05 | -1.32 | 111.0 | 115.3 | 88.3 | 72.8 | 78.5 | 86.5 |
| Ubiquitin carboxyl-terminal hydrolase 5 | UBP5_HUMAN | 96 kDa | 0.0066 | Infinite | 0.0 | 0.0 | 0.0 | 2.8 | 3.7 | 1.8 |

**Supplemental Table 6. Primer sequences of rat ADAMTS-7 (rTS7) fragments for mammalian two-hybrid.**

| Primer | Sequence(5'to3') |
|---|---|
| rTS7(26-246) forward | ACGGTCGACTTCCAACTGAGGGCCGGGCGGGTTTG |
| rTS7(26-246) reverse | TGCTCTAGAGCTCTCCACTTGCGGCTGCCCGTGGTA |
| rTS7(238-711) forward | ACGGTCGACTTTACCACGGGCAGCCGCAAGTGGAG |
| rTS7(238-711) reverse | TGCTCTAGACTCCTCAATGAGAATCTCTCGGGCTCC |
| rTS7(703-1007) forward | ACGGTCGACTTGGAGCCCGAGAGATTCTCATTGAG |
| rTS7(703-1007) reverse | TGCTCTAGAGACCGGCTGGTGCGGGTCGAAGTCAAC |
| rTS7(999-1595) forward | GCAGATATCAGTTGACTTCGACCCGCACCAGCCG |
| rTS7(999-1595) reverse | TGCTCTAGAGGGACATGAGCGGCAGCACTGAGCGCG |

# Supplemental Table 6

**Supplemental Figures and Figure Legends**

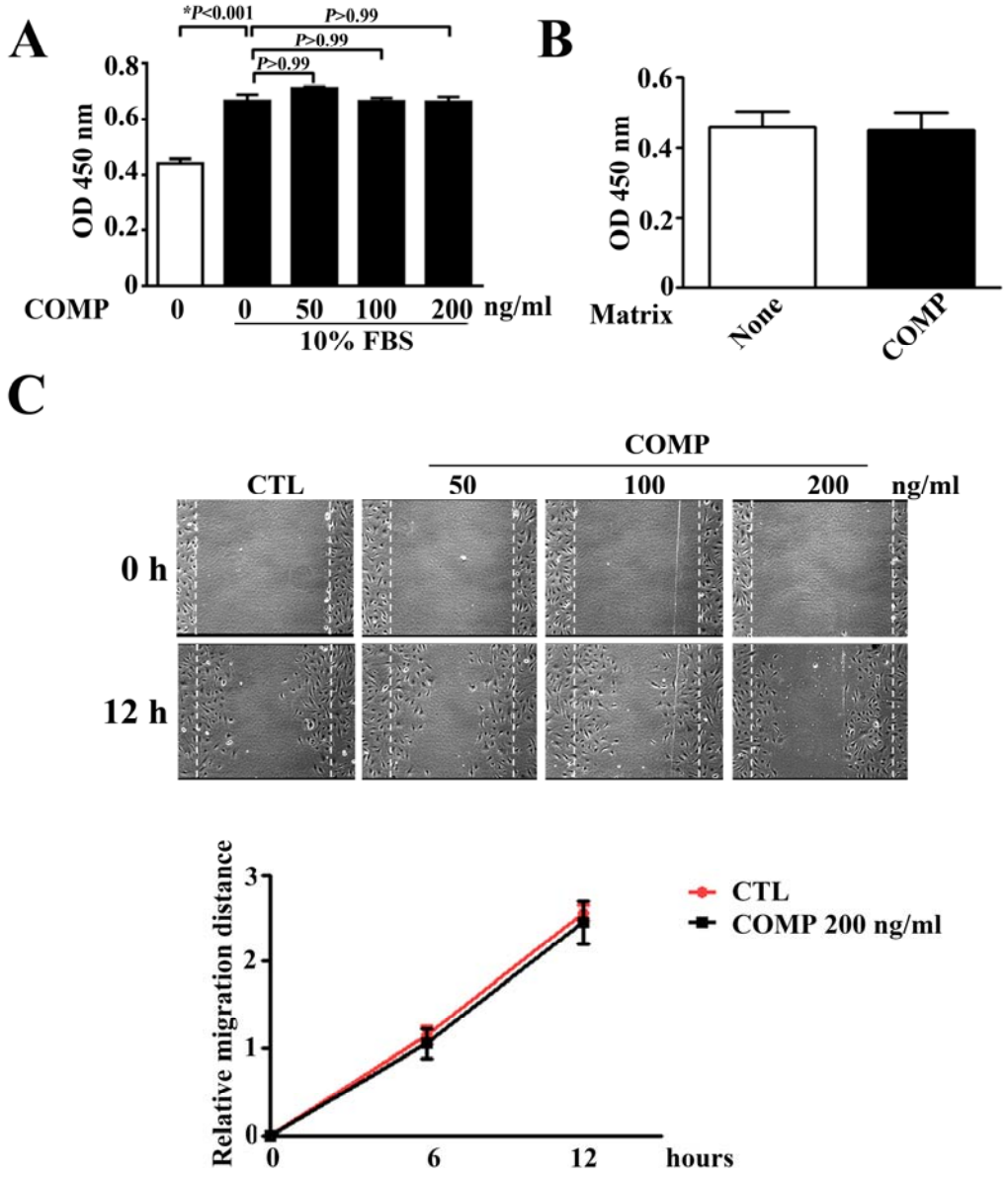



# Supplemental Figure 1

**Supplemental Figure 1. Characterization of ADAMTS-7 neutralization antibody.**

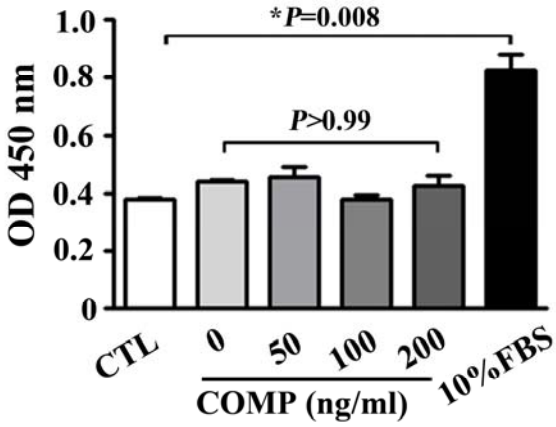
(A) Representative Western Blot analysis of COMP cleavage. COMP-stable transfected 293A cell were infected by Ad-ADAMTS-7, followed by increasing amount of ADAMTS-7

neutralization antibody. Full-length COMP band (110 KD under reducing condition) and a COMP fragment (≈55 KD) were detected. (B) Representative images of HA VSMC migration after scratch would injury in the presence or absence of ADAMTS-7 neutralization antibody. The mean distance migrated by VSMCs was quantified. Results are means±SEM from 3 independent experiments, **P*<0.05. Magnification is ×100.

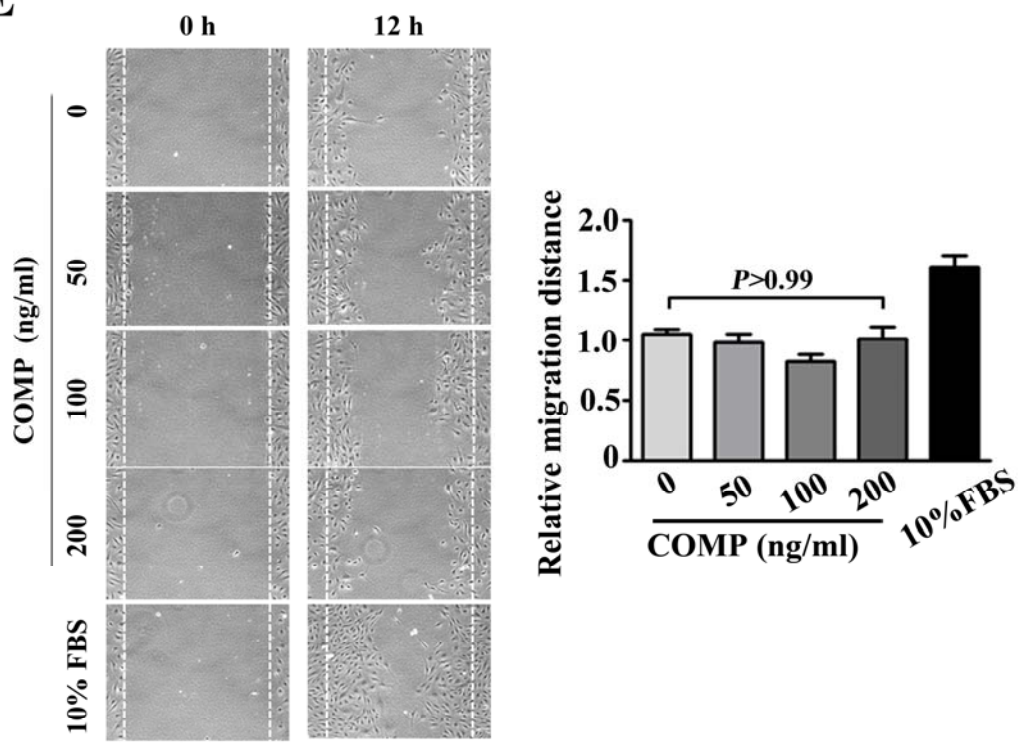



# Supplemental Figure 2

**D**

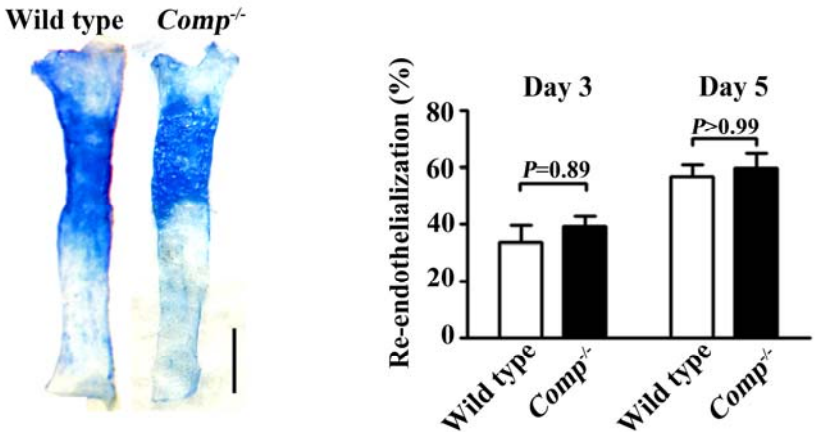



**E**

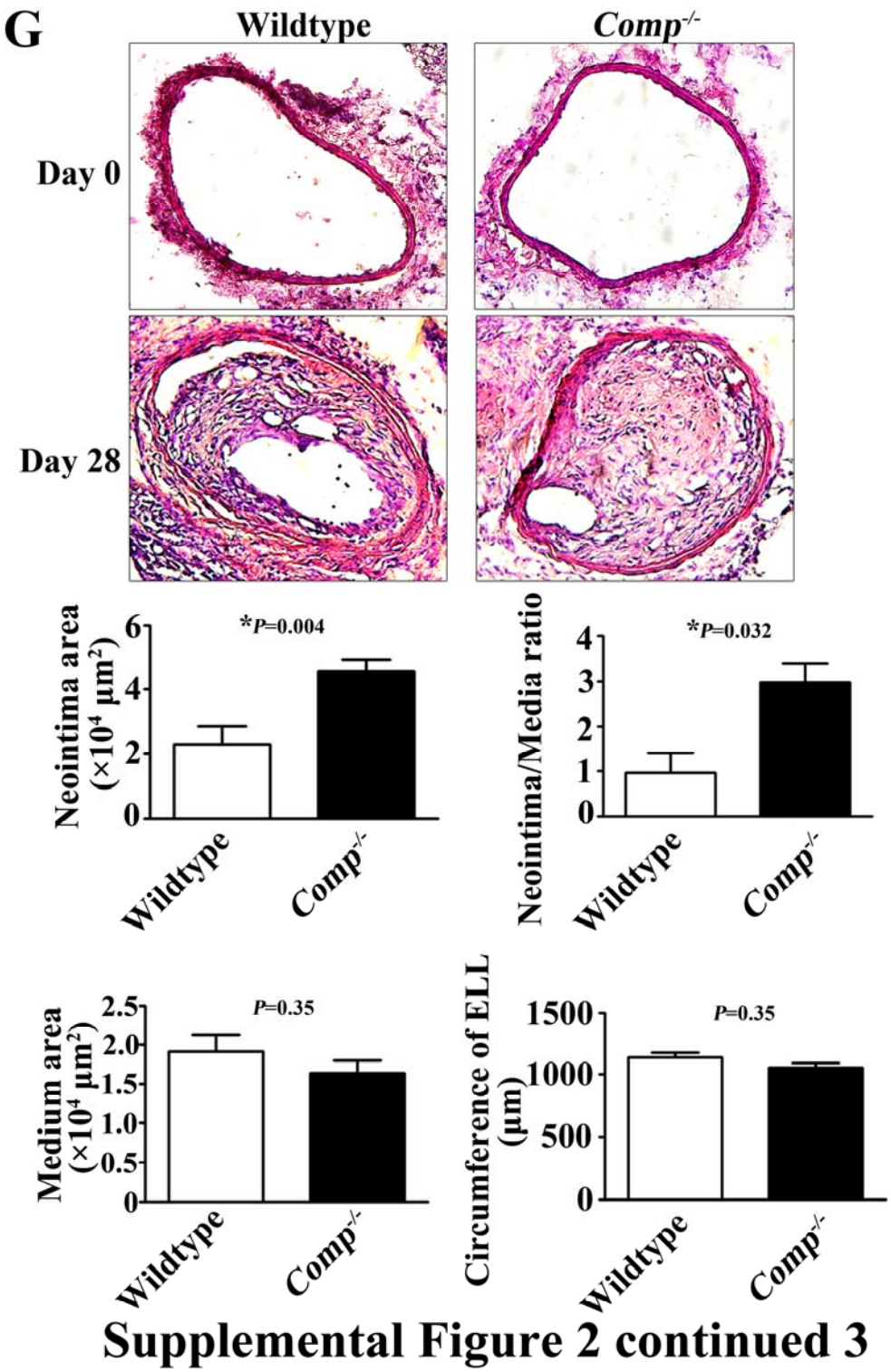



**Supplemental Figure 2 continued**

**F**




# Supplemental Figure 2 continued 2

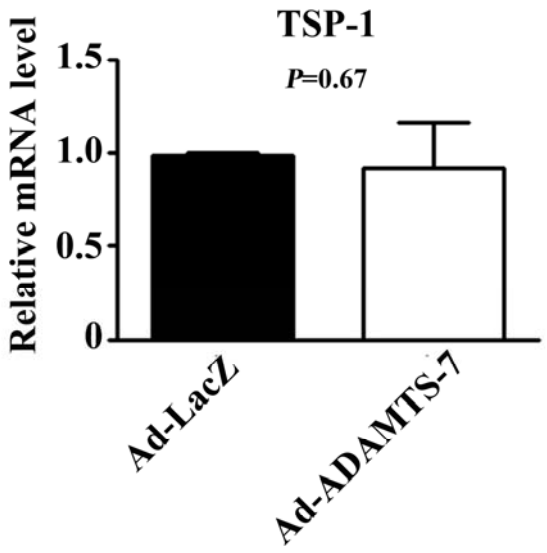



**Supplemental Figure 2 continued 3**

**Supplemental Figure 2. Effects of COMP on HUVEC proliferation and migration.**

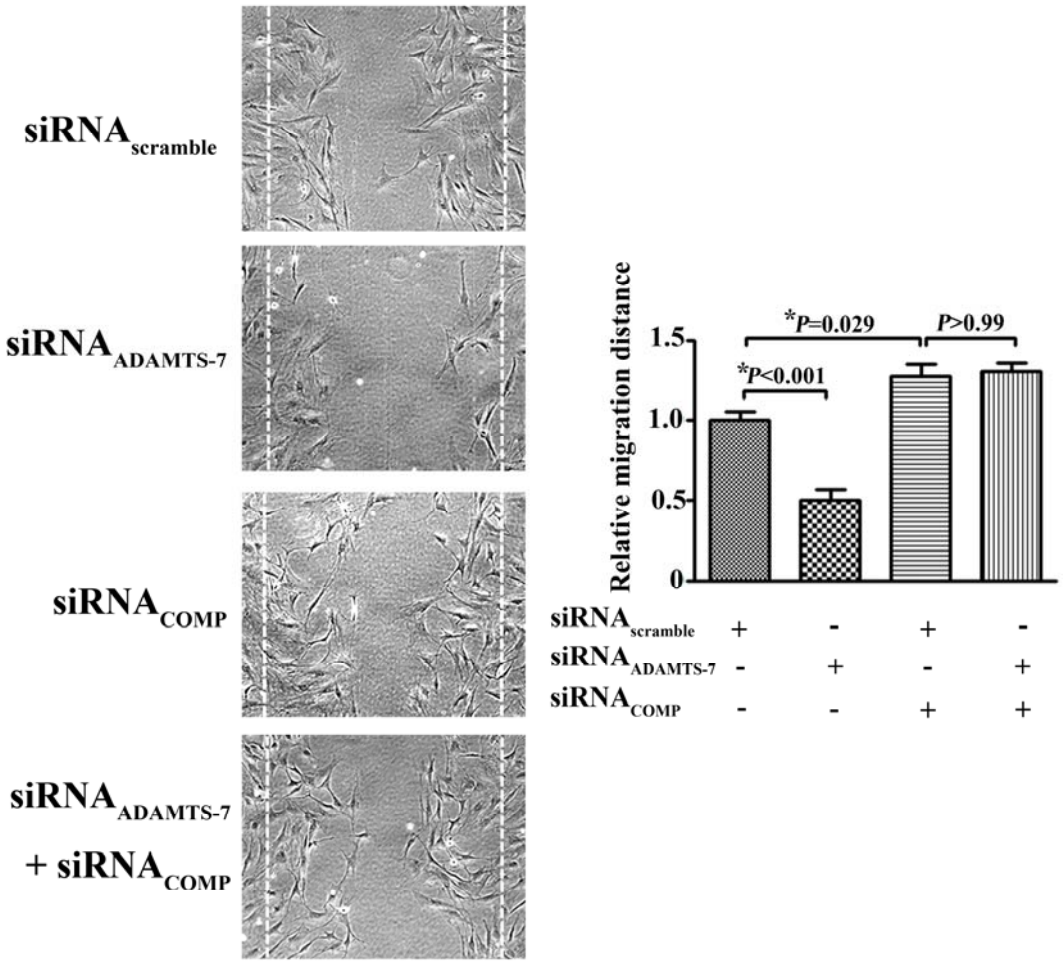
(A) Proliferation of HUVECs with COMP supplement in culture medium via cell counting kit-8. Results are means±SEM from 3 independent experiments performed in duplicate. (B)

HUVECs were cultured on a plate coated with or without COMP protein (30 μg/ml). Cell proliferation was analyzed 24 hours after synchronization. Results are means±SEM from 3 independent experiments. (C) Representative images of cell migration after scratch in COMP-treated HUVECs. The mean distance was quantified. Results are means±SEM from 3 independent experiments. Magnification was ×100. (D&E) Proliferation and migration of HUVECs with COMP under 1% FBS. 10% FBS was a positive control. Results were means±SEM from 3 independent experiments, **P*<0.05. (F) Representative pictures of Evans blue stained carotid arteries 3 or 5 days after vascular injury. Scar bar, 1 mm. n=6-8 for each group. (G) Neointima formation was determined on hematoxylin and eosin–stained cross sections of carotid arteries 0 day and 28 days after vascular wire injury in wild type and *Comp*$^{-/-}$ mice (n=6 each group). Scar bar, 100 μm. **P*<0.05.

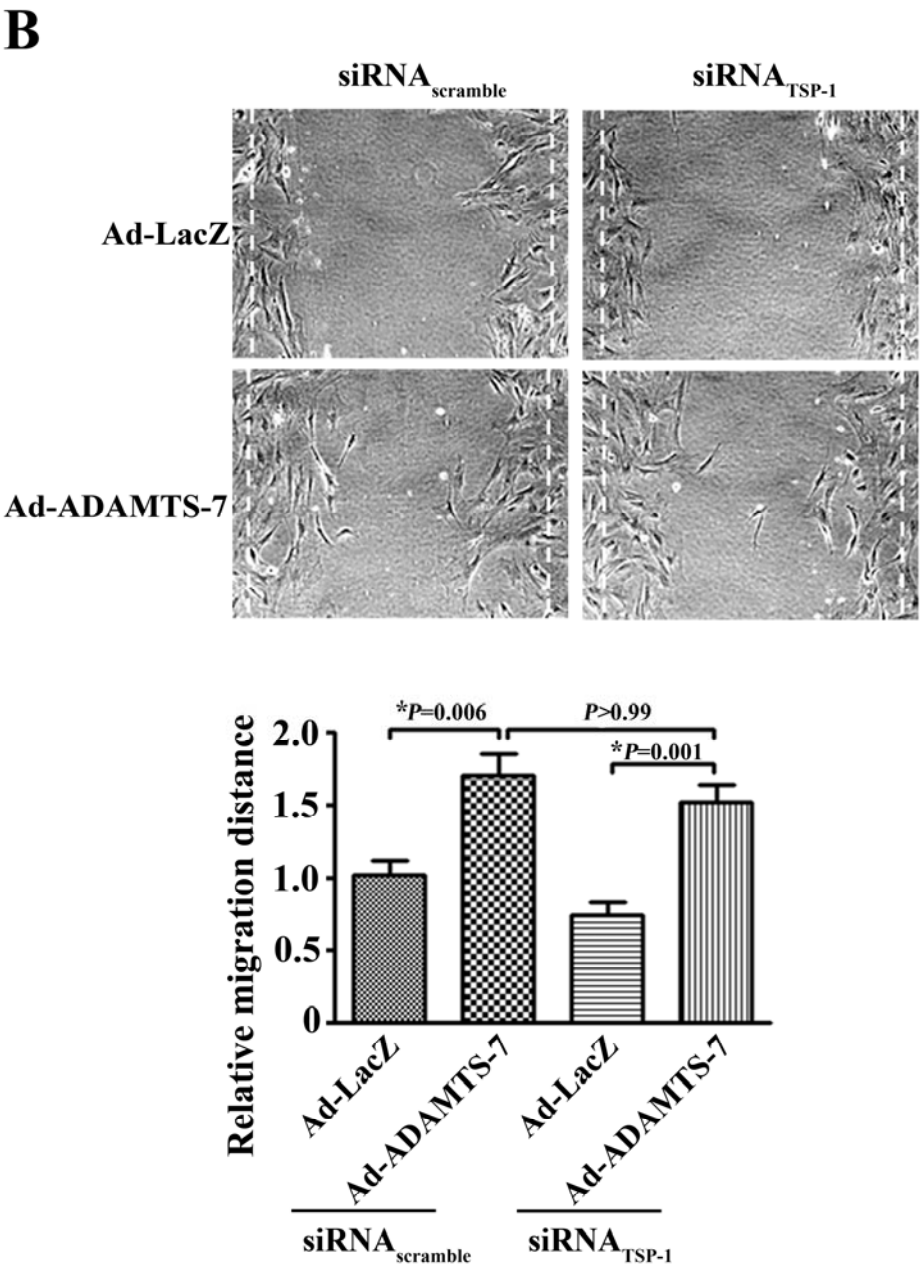



# Supplemental Figure 3

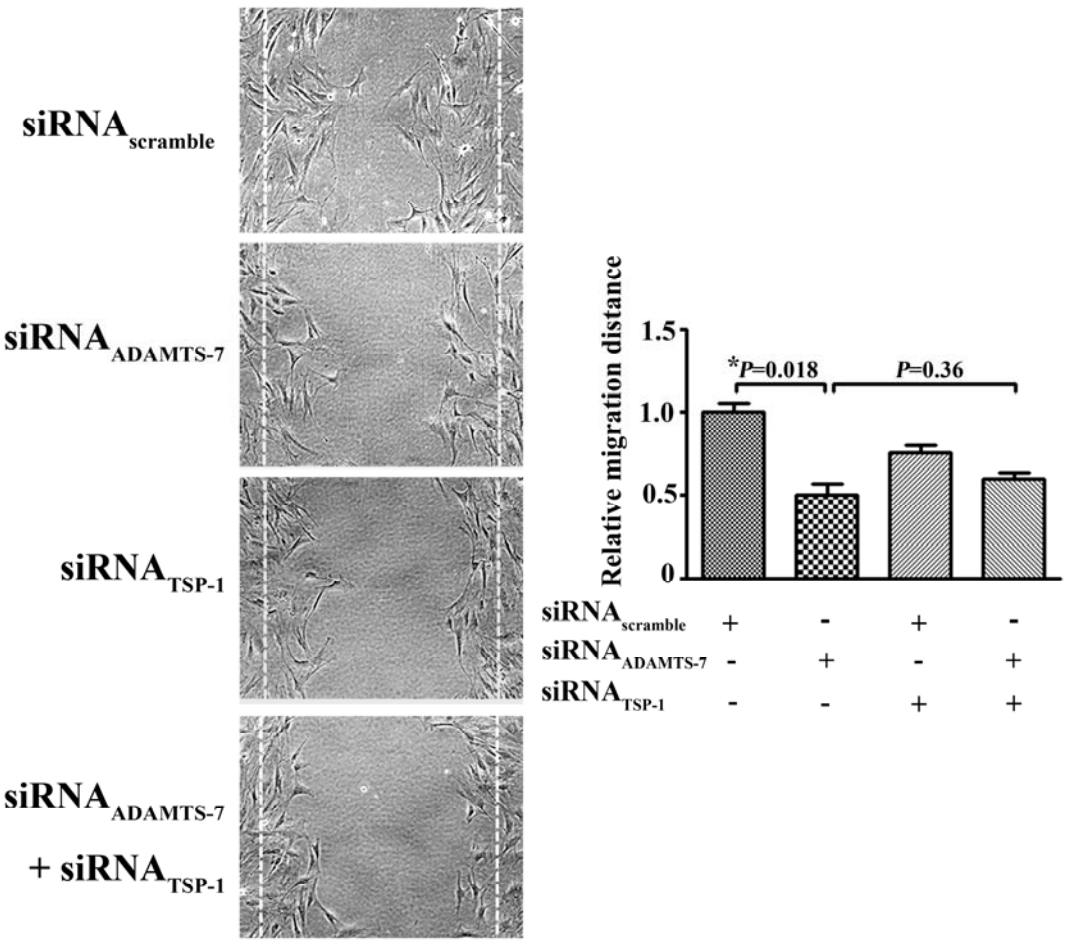
**Supplemental Figure 3. mRNA level of TSP-1 in Ad-*ADAMTS-7*-infected or Ad-*LacZ*-infected HUVECs.** Results are means±SEM from 5 independent experiments.




# Supplemental Figure 4



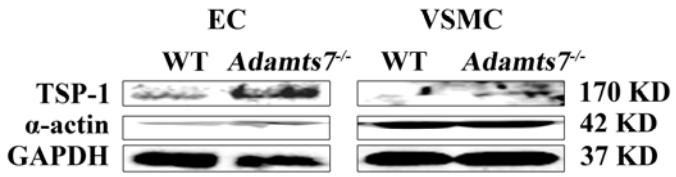

**Supplemental Figure 4 continued**




# Supplemental Figure 4 continued 2

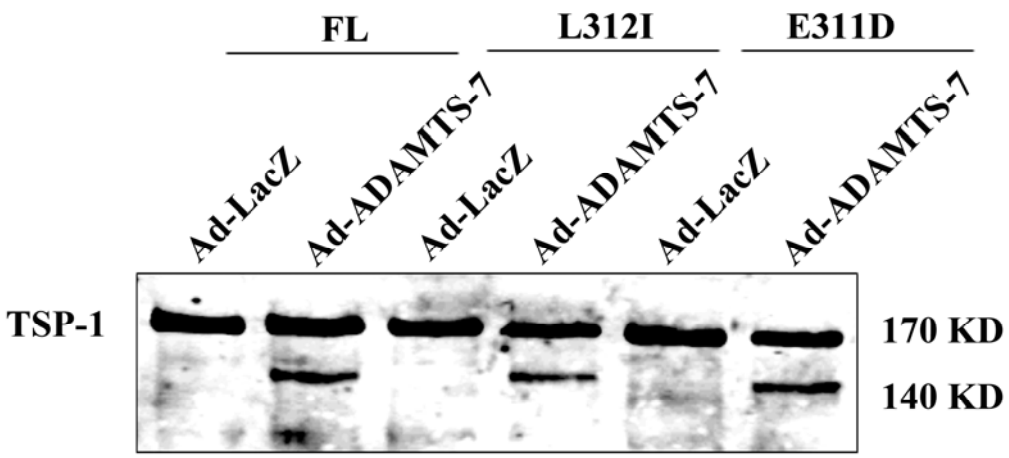
**Supplemental Figure 4. ADAMTS-7 mediates VSMCs migration via COMP but not TSP-1.** (A) Representative images of cell migration 12 hours after scratch in specific siRNA-treated rat VSMCs. Magnification was ×100. (B&C) Effect of ADAMTS-7 overexpression/silencing on cell migration in TSP-1 siRNA-treated rat VSMCs.

Magnification was ×100. The mean distance was quantified. Results are means±SEM from 3 independent experiments, *$P$<0.05.




# Supplemental Figure 5

**Supplemental Figure 5. Protein expression of TSP-1 in EC and VSMC from wildtype (WT) and *Adamts7*$^{-/-}$ aorta respectively.**




# Supplemental Figure 6

**Supplemental Figure 6. Western blot of TSP-1 cleaved by ADAMTS-7, mutant ADAMTS-7 L312I and E311D.**

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**APPENDIX X:**

**[§]German Mouse Clinic, Helmholtz Zentrum München, German Research Center for Environmental Health GmbH, Neuherberg, Germany**

Thure Adler [1,2]

Dirk H. Busch [2]

Antonio Aguilar-Pimentel[1,3]

Markus Ollert[3, 11]

Oana Amarie[1,4]

Tobias Stoeger[1,4]

Ali Önder Yildrim[1,4]

Oliver Eickelberg[4]

Cornelia Prehn[1]

Jerzy Adamski[1]

Lore Becker[1,5]

Alexandra Vernaleken[1, 5]

Thomas Klopstock [5,16,20,21]

Marion Horsch[1]

Johannes Beckers[1,18,19]

Kristin Moreth[1]

Raffi Bekeredjian[6]

Hugo Katus[6]

Lillian Garrett[1,8]

Sabine M. Hölter [1,8]

Annemarie Zimprich[1,8]

Wolfgang Wurst [8,14,15,16,17,21]

Oliver Puk[1,8]

Jochen Graw [8]

Wolfgang Hans[1]

Jan Rozman [1,19]

Martin Klingenspor[9,10]

Laura Brachthäuser[1,7]

Julia Calzada-Wack[1,7]

Dirk Janik[1,7]

Tanja Klein-Rodewald[1,7]

Frauke Neff[1,7]

Ildikó Rácz[1,12]

Andreas Zimmer[12]

Birgit Rathkolb [1,13,19]

Eckhard Wolf [13]

Manuela Gegenfurtner[1]

Ralph Steinkamp[1]

Christoph Lengger[1]

Holger Maier[1]

Claudia Stoeger[1]

Stefanie Leuchtenberger[1]

Valérie Gailus-Durner [1]

Helmut Fuchs [1]

Martin Hrabě de Angelis [1,18,19]

1 German Mouse Clinic, Institute of Experimental Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health GmbH, Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany

2 Institute for Medical Microbiology, Immunology and Hygiene, Technical University of Munich, Trogerstrasse 9, 81675 Munich, Germany

3 Department of Dermatology and Allergy, Biederstein, Klinikum rechts der Isar, Technische Universität München (TUM), Biedersteiner Str. 29, 80802 Munich,

4 Comprehensive Pneumology Center, Institute of Lung Biology and Disease, Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Ingolstädter Landstraße 1, 85764 Neuherberg, Germany and Member of the German Center for Lung Research

5 Department of Neurology, Friedrich-Baur-Institut, Ludwig-Maximilians-Universität München, Ziemssenstrasse 1a, 80336 Munich, Germany

6 Department of Cardiology, University of Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany

7 Institute of Pathology, Helmholtz Zentrum München, German Research Center for

Environmental Health GmbH, Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany

8 Institute of Developmental Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health GmbH, Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany

9 Chair for Molecular Nutritional Medicine, Technische Universität München, Else Kröner-Fresenius Center for Nutritional Medicine, 85350 Freising, Germany

10 ZIEL – Center for Nutrition and Food Sciences, Technische Universität München, 85350 Freising, Germany

11 Clinical Research Group Molecular Allergology, Center of Allergy and Environment Munich (ZAUM), Technische Universität München (TUM), and Institute for Allergy Research, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany

12 Institute of Molecular Psychiatry, University of Bonn, Sigmund-Freud-Strasse 25, 53127 Bonn, Germany

13 Ludwig-Maximilians-Universität München, Gene Center, Institute of Molecular Animal Breeding and Biotechnology, Feodor-Lynen Strasse 25, 81377 Munich, Germany

14 Chair of Developmental Genetics, Center of Life and Food Sciences Weihenstephan, Technische Universität München, Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany

15 Max Planck Institute of Psychiatry, Kraepelinstr. 2-10 , 80804 Munich, Germany

16 Deutsches Institut für Neurodegenerative Erkrankungen (DZNE) Site Munich, Schillerstrasse 44, 80336 Munich, Germany

17 Munich Cluster for Systems Neurology (SyNergy), Adolf-Butenandt-Institut, Ludwig-Maximilians-Universität München, Schillerstrasse 44, 80336 Munich, Germany

18 Chair of Experimental Genetics, Center of Life and Food Sciences Weihenstephan, Technische Universität München, Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany

19 Member of German Center for Diabetes Research (DZD), Ingolstaedter Landstraße 1, 85764 Neuherberg, Germany

20 German Network for Mitochondrial Disorders (mitoNET)

21 German Center for Vertigo and Balance Disorders, Munich, Germany

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# ADAMTS-7 Inhibits Re-Endothelialization of Injured Arteries and Promotes Vascular Remodeling Via Cleavage of Thrombospondin-1

Thorsten Kessler, Lu Zhang, Ziyi Liu, Xiaoke Yin, Yaqian Huang, Yingbao Wang, Yi Fu, Manuel Mayr, Qing Ge, Qingbo Xu, Yi Zhu, Xian Wang, Kjestine Schmidt, Cor de Wit, Jeanette Erdmann, Heribert Schunkert, Zouhair Aherrahrou and Wei Kong
for the German Mouse Clinic Consortium

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# SUPPLEMENTAL MATERIAL.

## Supplemental Methods

### Materials

Antibody against ADAMTS-7 was purchased from Abcam (Cambridge, UK). Antibodies against Thrombospondin-1 (TSP-1) were purchased from Neomarkers (Fremont, CA). Antibodies against BrdU were purchased from Sigma-Aldrich (St. Louis, MO). The antibody against GAPDH was purchased from Cell Signaling Technology (Boston, MA). The antibody against vWF was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The antibodies against Cyclin D1 and E1 were purchased from Bioworld (Minneapolis, MN). The neutralizing antibody of ADAMTS-7 (Antigen: LEDEEKDLKITH-KLH) was purchased from Biosynthesis Biotechnology Co., Ltd. (Beijing, China). Recombinant human TSP-1 was purchased from R&D Systems (Minneapolis, MN).

### Generation of *Adamts7*$^{-/-}$ mice

The *Adamts7* knockout embryonic stem cell (*Adamts7*-KO-ESC) line (EPD0209) was purchased from the European Conditional Mouse Mutagenesis Program (EUCOMM)[1]. Microinjection of *Adamts7*-KO-ESC into C57BL/6N blastocysts was achieved at the Knock Out Mouse Project (KOMP) repository at the University of California, Davis, USA[2]. The *Adamts7* targeting vector includes the insertion of a neomycin selection cassette and two loxP sites flanking exon 5 and exon 6 in the *Adamts7* genomic sequence. The *Adamts7* gene was interrupted by introducing an internal ribosome entry site followed by the beta-galactosidase sequence between exons 4 and 5. Male chimeras were obtained and backcrossed to C57BL/6

females in our animal facility to generate founders, which were then selected and genotyped using polymerase chain reaction (PCR) on genomic DNA isolated from ear punch biopsies. Heterozygous mice were intercrossed to generate knockout (*Adamts7*$^{-/-}$), heterozygous (*Adamts7*$^{+/-}$) and wildtype (WT) littermates. Genomic DNA was isolated from ear punches using standard methods. Tissue sections from mice were explanted, snap-frozen in liquid nitrogen and stored at -80°C until use. Homogenization and RNA-isolation were performed using TRIzol (Life Technologies) according to the manufacturer's recommendations. RNA was stored at -80°C until use. cDNA was generated using M-MLV Reverse Transcriptase (Life Technologies) and pdN6-Primers. Amplification of DNA and cDNA was performed using rTaq (GE Healthcare) with the recommended supplements. PCR-products were visualized on agarose-gels.

**X-gal staining of cryosections**

Organs were excised and embedded in Tissue Tek (Sakura), snap frozen in liquid nitrogen, and stored at -20°C until use. The tissues were sectioned into 8-10 µm cryosections. For X-gal staining, the cryosections were air dried, incubated in PBS that contained 0.5% glutaraldehyde at 4°C for 10 min, washed in PBS and incubated with X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) solution at 37°C overnight. Afterwards, the cryosections were washed in PBS, dehydrated and mounted using cover slips (Fisher).

**Large-Scale phenotyping of *Adamts7*$^{-/-}$ mice**

All animal studies followed the guidelines of the Animal Care and Use Committees of

Peking University, People's Republic of China, Schleswig-Holstein and Bavaria, Germany. Fifty-four mice, i.e., 29 females (15 WT, 14 *Adamts7*$^{-/-}$) and 25 males (10 WT, 15 *Adamts7*$^{-/-}$), were generated by intercrossing heterozygous *Adamts7*$^{+/-}$-mice and transferred to the German Mouse Clinic (GMC). At the GMC mice were maintained in IVC cages (Ventirack, Biozone, UK) with water and standard mouse chow (Altromin no. 1324) according to the GMC housing conditions and German laws.The mice were processed by standardized screening procedures as described previously[3, 4]. Briefly, the mice were characterized regarding morphology, behavior, neurology, eye morphology and function, nociception, energy metabolism, clinical chemistry/ hematology, immunology, allergies, steroid metabolism, cardiovascular function, lung function, and pathology.

**Wire-injury of mouse carotid artery**

Wire-injury of the mouse carotid artery was performed in 12-week-old mice as described by Lindner et al[5]. Through a middle line neck incision on the ventral side, the left common carotid artery (LCCA), including bifurcation, was exposed and cleaned from the surrounding tissue. Bulldog clamps were placed around the LCCA proximal to the aortic arch and the left internal carotid artery (LICA) for temporary control of blood flow; a 6-0 suture was placed around the left external carotid artery (LECA). An incision hole was made in the LECA, then a flexible wire (0.38 mm) was introduced into the LCCA by approximately 5 mm and passed 3 times toward and forth with rotation. The wire was removed, and the LECA was then tied off proximally. The skin incision was closed with surgical sutures. The area of remaining denudation at 3, 5 and 7 days after injury was determined by left ventricle injection of Evans

blue dye; quantification of the dye-stained area by blinded image analysis was performed as described previously[6]. Fourteen and 28 days after injury, the mouse arteries were harvested and embedded in Tissue Tek OCT (Sakura Finetek, Staufen, Germany); 6 μm serial cryostat sections were obtained from the bifurcation point and analyzed by matoxylin/eosin staining and Spot Image software (Diagnostic Instruments, Australia). All surgical studies followed the guidelines of the Animal Care and Use Committee of Peking University.

**BrdU incorporation**

BrdU incorporation was performed 3 days after injury as described previously[7]. Briefly, BrdU was administered to mice via an intraperitoneal injection at 48, 24, and 2 hours prior to sacrifice. The carotid arteries were then harvested and incubated with anti-BrdU and anti-vWF antibodies to perform dual immunofluorescence staining.

**Femoral artery injury in mice**

Thrombospondin-1 deficient (*Tsp1*$^{-/-}$) mice were purchased from Jackson Lab. A wire-mediated vascular injury was induced in the femoral arteries of *Tsp1*$^{-/-}$ or WT mice by an angioplasty guide wire as described previously[8]. Briefly, the femoral arteries were exposed by a longitudinal groin incision and monitored under a surgical microscope. The femoral artery was temporarily clamped at the level of the inguinal ligament, and an incision hole was created distal to the deep femoral branch. After release of the clamp, a 0.38-mm guide wire was advanced once by approximately 5 mm and was left in place for 1 minute to denude and dilate the artery. The wire was then removed, and the arteriotomy site was ligated with an 8-0

suture. For *in vivo* overexpression, a single exposure of $5\times10^8$ plaque forming units (pfus) of Ad-ADAMTS7 or Ad-LacZ adenovirus were delivered to the wire-injured femoral artery segments. The skin incision was closed with a 6-0 silk suture. The animals were monitored as per usual after surgery. ADAMTS-7 overexpression *in vivo* was confirmed by immunohistochemistry 3 days after adenovirus delivery (data not shown). Re-endothelialization of the femoral artery was determined by Evans blue staining 5 days after wire injury in mice.

**Immunohistochemistry and Dual Immunofluorescence**

To confirm ADAMTS-7 overexpression in femoral arteries, frozen sections of carotid arteries were incubated with rabbit primary anti-ADAMTS-7 antibody (Abcam, Cambridge, UK), horseradish peroxidase-conjugated goat anti-rabbit IgG and 3, 3-diaminobenzidine, successively. The sections were then counterstained with hematoxylin. For dual immunofluorescence, the frozen sections were first incubated with the antibodies mouse anti-BrdU (1:200) and rabbit anti-vWF (1:50) and then the secondary TRITC-conjugated goat anti-rabbit IgG (1:300) and FITC-conjugated goat anti-mouse IgG (1:300) (Rockland Inc. Gilbertsville, PA), respectively. Fluorescence was detected by confocal laser scanning microscopy (Leica, Germany).

**Cell culture**

HUVECs were isolated from human umbilical veins by type I collagenase (100 IU/ml) and by the differential attachment rate from other cells[9]. Human umbilical cords were

obtained from Peking University Third Hospital. The experiment was approved by the Ethics Committee of the Peking University Health Science Center, and it was conducted after informed consent was provided by the infants' parents. Cells were cultured in medium 199 (Gibco) that contained 10% fetal bovine serum (FBS, Hyclone), 4.17 mg/L recombinant human endothelial cell growth factor (Sigma), 1.4 IU/ml heparin sodium (Sigma), 3.0 mg/L thymidine (Sigma), 5.96 g/L HEPES, 2.2 g/L $NaHCO_3$, 200 U/mL penicillin, and 100 U /mL streptomycin and passaged by 0.05% trypsin digestion. HUVECs of passage 5–6 were used for experiments.

**Real-Time Quantitative PCR and Western Blot Analysis**

Real-time PCR amplification involved the use of an Mx3000 Multiplex Quantitative PCR System (Stratagene Corp, La Jolla, CA) and SYBR Green I reagent normalized to that of the internal control β-actin. The specific primers for human ADAMTS-7 were sense, 5’-GTGGAGACCCTGGTAGTAGC-3’, and antisense, 5’-TCTGCGTGGTGCGTGATCTTTA-3’. The primers for human Thrombospondin-1 were sense, 5’-GACTCCTAGAACGTGCGACCT-3’, and antisense, 5’-CATACAATCGTCTCGGGTATGC-3’, and the primers for human β -actin were sense, 5’-ATCTGGCACCACACCTTC-3’, and antisense, 5’-AGCCAGGTCCAGACGCA-3’. All amplification reactions were conducted over 40 cycles (an initial stage of 7 min at 94°C, then a three-step program of 30 s at 94°C, 30 s at 58°C and 30 s at 72°C) and were performed in duplicate.

Extracts that contained equal amounts of total protein were resolved by 10% or 6-20%

gradient SDS-PAGE. The membranes were incubated with primary antibody and IRDye 700DX-conjugated secondary antibodies (Rockland Inc., Gilbertsville, Pa). The immunofluorescence signal was detected by the Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NE).

**Cell proliferation assay**

After infected with Ad-LacZ or ADAMTS-7 for 48h, HUVECs were trypsinized to single-cell suspension, and 3000 cells in M199 that contained 10% FBS were transferred to each well of a 96-well plate. Cell Counting Kit-8(CCK-8) reagent was added 24 hours after synchronization and incubated at 37°C for 2 to 4 hours according to the color change. The OD (optical density) value at 450 nm was read by a microplate reader (Varioskan Flash, Thermo Fisher). For cell cycle analyses, HUVECs were fixed with 70% ethanol and then stained with 20 μg/mL propidium iodide (PI) and 500 mg/mL RNase A (Sigma), followed by FACS analysis. Each experiment was performed a minimum of 3 times independently.

**Cell migration assay**

HUVECs were infected with Ad-LacZ or ADAMTS-7 for 48h before sratching assay and the modified Boyden Chamber analysis. For the scratching assay, HUVECs ($3 \times 10^5$ cells) were seeded in 6-well plates. The medium was changed to serum-free OPTI-MEM for synchronization after adenoviral infection. Six hours later, scratching was made, and fresh medium that contained 10% FBS was added. Four fields were randomly selected in each well

to record gap distances immediately following scratching at 12, 18 and 24 hours to calculate cell migration.

A modified Boyden Chamber (Chemicon International, MA) coated with an 8-μm barrier of collagen type I was used to test the HUVECs migration ability. For this, 200 μl of suspended HUVECs ($2 \times 10^5$/ml in M199 that contained 10% FBS) were placed in the upper chamber. The lower chamber contained PDGF-BB (20 ng/ml) as a chemoattractant. After 6, 9 or 12 hours, cells on the upper surface were removed by gentle abrasion with the use of a cotton bud, and cells on the underside (invaded cells) were fixed and stained with crystal violet. The mean number of cells on the lower surface was counted from 4 randomly chosen high-power fields (×100) under light microscopy in 3 independent experiments.

**Gel-LC-MS analysis of secretome**

Analysis of the secretome was performed as described[10]. Rat VSMCs were infected with adenovirus that contained GFP or ADAMTS-7. Conditioned media were precipitated with acetone and denatured with 2× SDS sample buffer (Invitrogen) at 97°C for 5 min. Proteins were separated on 4%-12% NuPAGE Bis-Tris gels (Invitrogen). After silver staining, each lane was cut into 16 gel bands without gaps and digested with trypsin (Promega) using a robotic digestor (ProGest, Digilab) overnight. Peptides were separated by nano-flow HPLC on a reverse-phase column (C18 PepMap 100, 3 μm, 100 Å, 25 cm; Thermo Fisher Scientific) and identified by a LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific). Spectra were acquired with the full MS scan range of m/z 450-2000 followed by six dependent MS2

scans using dynamic exclusion. The results were blasted against the UniProt/SwissProt database (SwissProt 57.15, 515203 entries) using Mascot server 2.3.01 (Matrix Science). The following parameters were used: peptide tolerance = 10 ppm, fragment tolerance = 0.8 Da, carbamidomethylation of cysteine as fixed modification, oxidation of methionine as variable modification, and 2 missed cleavages were allowed. Scaffold (version 3.6.2, Proteome Software) was used to validate MS/MS-based peptide and protein identification with the following filters: peptide probability > 95%, protein probability > 99%, and minimum no. peptides per protein ≥ 2.

**Co-Immunoprecipitation**

Cells or rat aorta artery lysates were incubated with anti-ADAMTS-7 or anti-TSP-1 antibodies prior to immunoprecipitation with protein P/A agarose beads (Santa Cruz, CA). The precipitated proteins were resolved by 10% SDS-PAGE and immunoblotted with anti-TSP-1 or anti-ADAMTS-7 antibodies, respectively. Rabbit or mouse IgG antibodies served as a negative control.

In another site of study, Eahy 926 cells were co-transfected with Flag-CMV vectors that encoded various ADAMTS-7 fragments (Flag-TS7(26-246), Flag-TS7(238-711), Flag-TS7(703-1007), and Flag-TS7(999-1595) and full length TSP-1 for 48 hours respectively. Cell lysates were incubated with anti-Flag antibody and then immunoblotted with anti-TSP-1 antibodies.

**TSP1 siRNA Transfection**

Small interfering RNA (siRNA) against human TSP-1 was purchased from GenePharma Co., Ltd (Shanghai). Sequences corresponding to the siRNA of TSP-1 were sense, 5'-GCGUGUUUGACAUCUUUGATT-3', and antisense, 5'-UCAAAGAUGUCAAACACGCTT-3'. Transfection of HUVECs with siRNA (20 nmol/L) *in vitro* was performed using RNAi MAX (Invitrogen). A scramble stealth RNAi duplex served as a negative control.

**Subcloning TSP-1 plasmid**

The cDNA fragment encoding the full-length human *TSP1* (NCBI Reference sequence: NM_003246.2) was cloned into the *SalI/XbaI* sites of pDNR-CMV and pFlag-CMV plasmid. The primer sequences were sense, 5'-TGCTCTAGAACAGGATCCCTGCTGGGCACCAACA-3', and antisense, 5'-CGGGGTACCTCCAGAAGGTGCAATACCAGCATTGG-3'.

**Mammalian two-hybrid assay**

The fragments that encoded the 4 functional domains of rat *ADAMTS-7* (i.e., the prodomain [TS7(26-246); aa 26-246], the metalloproteinase plus disintegrin-like and cysteine-rich domain [TS7(238-711); aa 238-711], the spacer-1 plus three TSP repeats [TS7(703-1007); aa 703-1007], and the spacer-2 plus four C-terminal TSP repeats [TS7(999-1595); aa 999-1595]) were amplified by PCR and subcloned in-frame into the *Sal*I/*Xba*I or *Ecor*V/*Xba*I sites of pACT (pACT-TS7(26-246), pACT-TS7(238-711), pACT-TS7(703-1007), and pACT-TS7(999-1595), respectively. cDNA inserts that encoded

human TSP-1 were subcloned in-frame into the pBIND vector to generate the indicated plasmids (pBIND-TSP-1). Eahy 926 cells were cotransfected with the target and bait constructs, together with the reporter plasmid pG5luc-luciferase at a ratio of 1:1:1. After 48 h, the transfected cells were harvested, and the cell lysates were used for a luciferase assay with the Dual-Luciferase Reporter Assay System (Promega). The fragment primer sequences are listed in Supplemental Table 6.

**Mutagenesis of TSP-1**

The ADAMTS-1 cleavage site in TSP-1 (glutamic acid 311 and leonine 312)[11] were mutated to Isoleucine and Asparagine respectively. Site-mutation was mediated by DpnI-Restriktionsendonuklease (Takara).

***In vitro* digestion of TSP1**

The digestion assay was performed as described previously[11]. Briefly, COS-7 cells were infected with adenovirus that expressed LacZ (control) or ADAMTS-7 for 48 hours. The medium was then changed to serum-free DMEM. The culture medium (CM) was collected after 24 h of incubation and concentrated by centrifugal filter devices (Amicon Ultra-0.5, Millipore). Purified hTSP-1 (R&D) was incubated with CM from adenoviral infected cells at 37°C for 4 hours.

**Cleavage of TSP-1 by recombinant catalytic domain of ADAMTS-7 *in vitro***

The bacterial expression vector pGEX-6p-1 was used to produce recombinant

glutathione S-transferase (GST) fusion proteins in *Escherichia coli*. The cDNA fragments that encoded a catalytic domain-containing segment of rADAMTS-7 (aa 217-427) were subcloned into the *BamHI/XhoI* site. The primer sequences were sense: 5'-CGCGGATCCTCAATCAGCAAAGAGAAGTG-3', and antisense: 5'-CCGCTCGAGGGACGGTCATCTAAGCACAG-3'. Purified hTSP-1 was incubated with the bacteria-expressed catalytic domain of ADAMTS-7 in a digestion buffer (50 mM Tris-HCl, 100 mM NaCl, 5 mM $CaCl_2$, 2 mM $ZnCl_2$, and 0.05% Brij-35, pH 7.5) at 37°C for 4 h[12].

**Isolation of the mouse aortic ECs and VSMCs.**

After PBS perfusion, the mouse arteries were harvested and dissected longitudinally. Endothelium was carefully scraped in PBS and collected from 7 mice by centrifugation. The precipitate was resuspended with 60 μl lysis buffer. For VSMC isolation, media of the aorta were tore up and grinded in lysis buffer. Expression of TSP-1 was analyzed with Western blot.

**Statistical Analysis**

All results were expressed as the mean ± standard error of the mean (SEM). Statistical analysis involved the use of Mann-Whitney U test for comparison of two groups to evaluate the effects of ADAMTS-7 on the BrdU incorporation, cell proliferation, and TSP-1 concentration by ELISA analysis in cell condition medium, to analyze the role of COMP on HUVEC proliferation, to assess the postinjury neointima area in WT and *comp*$^{-/-}$ mice. Comparisons among more than 2 groups involved non-parametric Kruskal-Wallis test with a

Dunn's post-hoc test to evaluate the effects of ADAMTS-7 neutralization antibody on cell proliferation and migration. Comparison of more than 2 groups involved two-way ANOVA followed by the Bonferroni test for post-hoc comparison as appropriate to evaluate the effects of ADAMTS-7 on re-endothelialization, neointima formation, the cell cycle and cell migration, as well as the effect of TSP-1 on ADAMTS-7 mediated cell proliferation, migration and re-endothelialization. Statistical analyses involved the use of GraphPad Prism 6.0 (GraphPad Software Inc, La Jolla, CA). All *P* -values were two-sided and a $P<0.05$ was considered statistically significant.

**Supplemental Table 1. Large-scale phenotyping of the *Adamts7*$^{-/-}$ mouse (in cooperation with the German Mouse Clinic).**

| Screens | Phenotype of *Adamts7*$^{-/-}$-mice |
|---|---|
| Behaviour | Decreased anxiety in open field test |
| Neurology | Reduced rotarod latency in females |
| Nociception | None |
| Dysmorphology | None |
| *Clinical Chemistry* | *Mild effects on triglycerides and red blood cell count* |
| *Energy Metabolism* | *Increased maximum oxygen consumption in males* |
| *Cardiovascular* | *None* |
| Eye | None |
| Immunology | None |
| Allergy | None |
| Steroid Metabolism | None |
| Lung function | Increase in lung function parameters, reduced resistance |
| *Pathology* | *None* |

# Supplemental Table 1

**Supplemental Table 2. Phenotyping of *Adamts7*$^{-/-}$ mice (KO) compared to WT mice regarding energy metabolism, clinical chemistry and hematology. Data are mean ± SD.**

| Test | Female | | Male | | Linear model | | |
|---|---|---|---|---|---|---|---|
| | | | | | sex | genotype | sex:genotype |
| **Energy metabolism** | **WT (n=7)** | **KO (n=8)** | **WT (n=7)** | **KO (n=8)** | | | |
| Avg. mass [g] | 21.8 ± 2.2 | 21.3 ± 1.1 | 28.1 ± 1 | 28.3 ± 1.6 | <0.001 | 0.77 | 0.556 |
| Food intake [g] | 1.8 ± 0.7 | 1.4 ± 0.8 | 2.1 ± 0.7 | 2.4 ± 0.5 | 0.571 | 0.996 | 0.176 |
| Max. VO2 [ml/(h animal)] | 115.57 ± 18.14 | 117.88 ± 9.42 | 119.86 ± 7.36 | 134.63 ± 16.14 | 0.002 | 0.014 | 0.261 |
| Avg. distance [cm] | 5928 ± 1270 | 6532 ± 1983 | 4837 ± 3106 | 4576 ± 1624 | 0.057 | 0.824 | 0.577 |
| | | | | | | | |
| **Clinical chemistry** | **WT (n=15)** | **KO (n=14)** | **WT (n=10)** | **KO (n=15)** | | | |
| Fasting glucose [mM] | 5.79 ± 0.75 | 6.3 ± 0.86 | 6.63 ± 0.86 | 7.16 ± 1.37 | 0.002 | 0.056 | 0.965 |
| Creatinine [µM] | 10.43 ± 1.57 | 91.3 ± 2.7 | 9.65 ± 1.37 | 10.99 ± 1.42 | 0.298 | 0.973 | 0.013 |
| Triglycerides [mM] | 0.98 ± 0.28 | 0.86 ± 0.17 | 1.80 ± 0.31 | 1.49 ± 0.26 | <0.001 | 0.003 | 0.177 |
| Cholesterol [mM] | 1.77 ± 0.26 | 1.69 ± 0.21 | 2.03 ± 0.28 | 1.98 ± 0.30 | <0.001 | 0.377 | 0.818 |
| ALP [U/l] | 146 ± 15 | 157 ± 11 | 84 ± 8 | 83 ± 10 | <0.001 | 0.137 | 0.06 |
| | | | | | | | |
| **Hematology** | **WT (n=15)** | **KO (n=14)** | **WT (n=10)** | **KO (n=15)** | | | |
| RBC [$10^6$/mm$^3$] | 10.85 ± 0.24 | 10.58 ± 0.39 | 10.77 ± 0.22 | 10.62 ± 0.36 | 0.844 | 0.02 | 0.503 |
| HGB [g/dl] | 16.51 ± 0.29 | 15.96 ± 0.53 | 16.13 ± 0.39 | 15.95 + 0.48 | 0.105 | 0.004 | 0.139 |
| WBC [$10^3$/mm$^3$] | 7.55 ± 2.04 | 6 ± 0.88 | 7.65 ± 1.5 | 8.79 ± 1.52 | 0.001 | 0.635 | 0.003 |
| PLT [$10^3$/mm$^3$] | 1281.6 ± 106.47 | 1316.79 ± 97.55 | 1331.8 ± 113.71 | 1291.6 ± 201.71 | 0.746 | 0.948 | 0.331 |

**Supplemental Table 3. Phenotyping of *Adamts7*$^{-/-}$ mice (KO) compared to WT mice regarding pulmonary function. Data are mean [CI].**

| Test | Female WT n=5 | Female KO n=6 | p-value |
|---|---|---|---|
| Tidal volume, ml | 0.22 [0.22-0.22] | 0.23 [0.23-0.24] | 0.011 |
| Vital capacity, ml | 0.98 [0.91-1.16] | 1.23 [1.19-1.50] | 0.061 |
| Funct. residual capacity, ml | 0.25 [0.23-0.25] | 0.32 [0.28-0.36] | 0.024 |
| Residual volume, ml | 0.015 [0.008-0.02] | 0.025 [0.015-0.0.033] | 0.4 |
| Total lung capacity, ml | 0.96 [0.89-1.17] | 1.26 [1.16-1.39] | 0.052 |
| Forced vital capacity, ml | 0.89 [0.81-1.08] | 1.24 [1.05-1.43] | 0.19 |
| Forced exspiratory volume, ml | 0.87 [0.79-1.04] | 1.195 [1.035-1.35] | 0.111 |
| Dynamic lung compliance, ml/cm$H_2O$ | 0.02 [0.02-0.02] | 0.03 [0.03-0.03] | 0.015 |
| Lung resistance, cm$H_2O$/ml/s | 1.36 [1.34-1.39] | 1.25 [1.22-1.29] | 0.126 |

**Supplemental Table 4. Blood lipid levels after 15 weeks of western diet comparing WT mice and *Adamts7*$^{-/-}$ mice (KO). Data are mean ± SD.**

| **Test** | **WT**<br>**n=8** | **KO**<br>**n=5** | **p-value** |
|---|---|---|---|
| Cholesterol, mmol/l | 5.17 ± 1.81 | 4.83 ± 1.16 | 0.721 |
| LDL-cholesterol, mmol/l | 0.53 ± 0.19 | 0.52 ± 0.09 | 0.916 |
| HDL-cholesterol, mmol/l | 2.10 ± 0.53 | 2.06 ± 0.41 | 0.879 |
| Triglycerides, mmol/l | 0.58 ± 0.12 | 0.60 ± 0.15 | 0.790 |

**Supplemental Table 5. Differentially expressed proteins in the conditioned media of Ad-*GFP* (G) and Ad-*ADAMTS-7* (T) SMCs.**

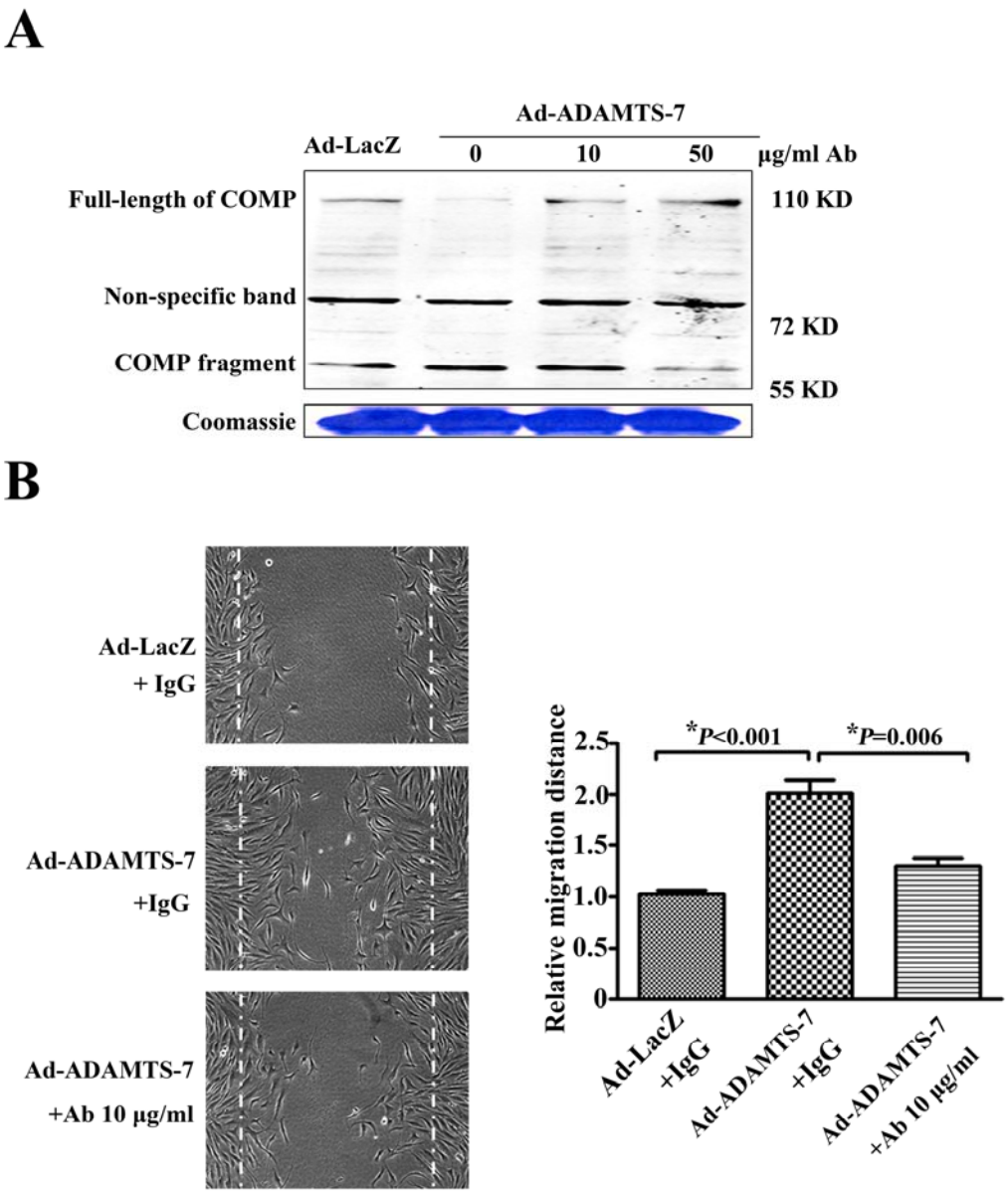
| Protein Name | UniProt ID | Mw | T-Test (*P*-Value) | Fold Change (T/G) | Normalized Spectral Count | | | Normalized Spectral Count | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | G1 | G2 | G3 | T1 | T2 | T3 |
| 6-phosphogluconate dehydrogenase, decarboxylating | 6PGD_RAT | 53 kDa | 0.00003 | Infinite | 0.0 | 0.0 | 0.0 | 5.5 | 6.4 | 6.4 |
| A disintegrin and metalloproteinase with thrombospondin motifs 7 | ATS7_RAT | 176 kDa | 0.00020 | Infinite | 0.0 | 0.0 | 0.0 | 152.9 | 189.0 | 150.2 |
| Actin-related protein 3 | ARP3_RAT | 47 kDa | 0.049 | 2.95 | 0.0 | 5.8 | 2.9 | 10.1 | 9.1 | 6.4 |
| Alpha-actinin-1 | ACTN1_RAT | 103 kDa | 0.016 | 1.41 | 30.5 | 34.9 | 30.7 | 45.1 | 50.2 | 40.1 |
| Alpha-actinin-4 | ACTN4_RAT | 105 kDa | 0.030 | 1.29 | 31.7 | 38.4 | 37.4 | 44.2 | 51.1 | 43.7 |
| Elastin | ELN_RAT | 73 kDa | 0.048 | -1.90 | 79.3 | 78.0 | 51.8 | 51.6 | 32.0 | 26.4 |
| Elongation factor 2 | EF2_RAT | 95 kDa | 0.027 | 2.26 | 4.9 | 4.7 | 3.8 | 12.9 | 10.0 | 7.3 |
| Growth/differentiation factor 6 | GDF6_RAT | 51 kDa | 0.017 | -Infinite | 8.5 | 4.7 | 3.8 | 0.0 | 0.0 | 0.0 |
| Guanine nucleotide-binding protein subunit beta-2-like 1 | GBLP_RAT | 35 kDa | 0.019 | 6.75 | 2.4 | 0.0 | 0.0 | 4.6 | 7.3 | 4.6 |
| Heterogeneous nuclear ribonucleoprotein K | HNRPK_RAT | 51 kDa | 0.0010 | Infinite | 0.0 | 0.0 | 0.0 | 4.6 | 6.4 | 4.6 |
| Heterogeneous nuclear ribonucleoprotein Q | HNRPQ_RAT | 60 kDa | 0.0020 | Infinite | 0.0 | 0.0 | 0.0 | 3.7 | 3.7 | 5.5 |
| Importin subunit beta-1 | IMB1_RAT | 97 kDa | 0.031 | Infinite | 0.0 | 0.0 | 0.0 | 2.8 | 6.4 | 2.7 |
| Low molecular weight phosphotyrosine protein phosphatase | PPAC_RAT | 18 kDa | 0.031 | Infinite | 0.0 | 0.0 | 0.0 | 2.8 | 6.4 | 2.7 |
| Moesin | MOES_MOUSE | 68 kDa | 0.0065 | 2.18 | 8.5 | 7.0 | 6.7 | 13.8 | 19.2 | 15.5 |
| Olfactomedin-like protein 3 | OLFL3_RAT | 46 kDa | 0.046 | -1.56 | 7.3 | 9.3 | 7.7 | 6.4 | 3.7 | 5.5 |
| Osteopontin | OSTP_RAT | 35 kDa | 0.023 | -5.80 | 20.7 | 17.5 | 9.6 | 2.8 | 5.5 | 0.0 |
| Periostin | POSTN_MOUSE | 93 kDa | 0.023 | -1.60 | 34.2 | 32.6 | 32.6 | 21.2 | 14.6 | 26.4 |
| Peroxiredoxin-5, mitochondrial | PRDX5_RAT | 22 kDa | 0.040 | 5.95 | 0.0 | 0.0 | 3.8 | 5.5 | 11.0 | 6.4 |
| Pigment epithelium-derived factor | PEDF_MOUSE | 46 kDa | 0.0066 | -1.49 | 32.9 | 31.4 | 29.7 | 19.3 | 19.2 | 24.6 |
| Proliferation-associated protein 2G4 | PA2G4_MOUSE | 44 kDa | 0.00064 | Infinite | 0.0 | 0.0 | 0.0 | 3.7 | 2.7 | 2.7 |
| Protein DJ-1 | PARK7_RAT | 20 kDa | 0.0057 | Infinite | 0.0 | 0.0 | 0.0 | 4.6 | 2.7 | 2.7 |
| Pyruvate kinase isozymes M1/M2 | KPYM_RAT | 58 kDa | 0.030 | 2.13 | 7.3 | 9.3 | 18.2 | 24.9 | 28.3 | 20.9 |
| Septin-11 | SEP11_RAT | 50 kDa | 0.012 | 7.94 | 0.0 | 0.0 | 2.9 | 6.4 | 6.4 | 10.0 |
| Sushi repeat-containing protein SRPX | SRPX_RAT | 52 kDa | 0.018 | -1.92 | 8.5 | 11.6 | 9.6 | 5.5 | 3.7 | 6.4 |
| Sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1 | SVEP1_RAT | 387 kDa | 0.0027 | -8.51 | 4.9 | 5.8 | 4.8 | 0.0 | 0.0 | 1.8 |
| T-complex protein 1 subunit theta | TCPQ_MOUSE | 60 kDa | 0.0014 | Infinite | 0.0 | 0.0 | 0.0 | 5.5 | 5.5 | 3.6 |
| Thioredoxin | THIO_RAT | 12 kDa | 0.027 | 3.04 | 0.0 | 4.7 | 2.9 | 8.3 | 8.2 | 6.4 |
| Thrombospondin-1 | TSP1_MOUSE | 130 kDa | 0.05 | -1.32 | 111.0 | 115.3 | 88.3 | 72.8 | 78.5 | 86.5 |
| Ubiquitin carboxyl-terminal hydrolase 5 | UBP5_HUMAN | 96 kDa | 0.0066 | Infinite | 0.0 | 0.0 | 0.0 | 2.8 | 3.7 | 1.8 |

**Supplemental Table 6. Primer sequences of rat ADAMTS-7 (rTS7) fragments for mammalian two-hybrid.**

| Primer | Sequence(5'to3') |
|---|---|
| rTS7(26-246) forward | ACGGTCGACTTCCAACTGAGGGCCGGGCGGGTTTG |
| rTS7(26-246) reverse | TGCTCTAGAGCTCTCCACTTGCGGCTGCCCGTGGTA |
| rTS7(238-711) forward | ACGGTCGACTTTACCACGGGCAGCCGCAAGTGGAG |
| rTS7(238-711) reverse | TGCTCTAGACTCCTCAATGAGAATCTCTCGGGCTCC |
| rTS7(703-1007) forward | ACGGTCGACTTGGAGCCCGAGAGATTCTCATTGAG |
| rTS7(703-1007) reverse | TGCTCTAGAGACCGGCTGGTGCGGGTCGAAGTCAAC |
| rTS7(999-1595) forward | GCAGATATCAGTTGACTTCGACCCGCACCAGCCG |
| rTS7(999-1595) reverse | TGCTCTAGAGGGACATGAGCGGCAGCACTGAGCGCG |

# Supplemental Table 6

**Supplemental Figures and Figure Legends**

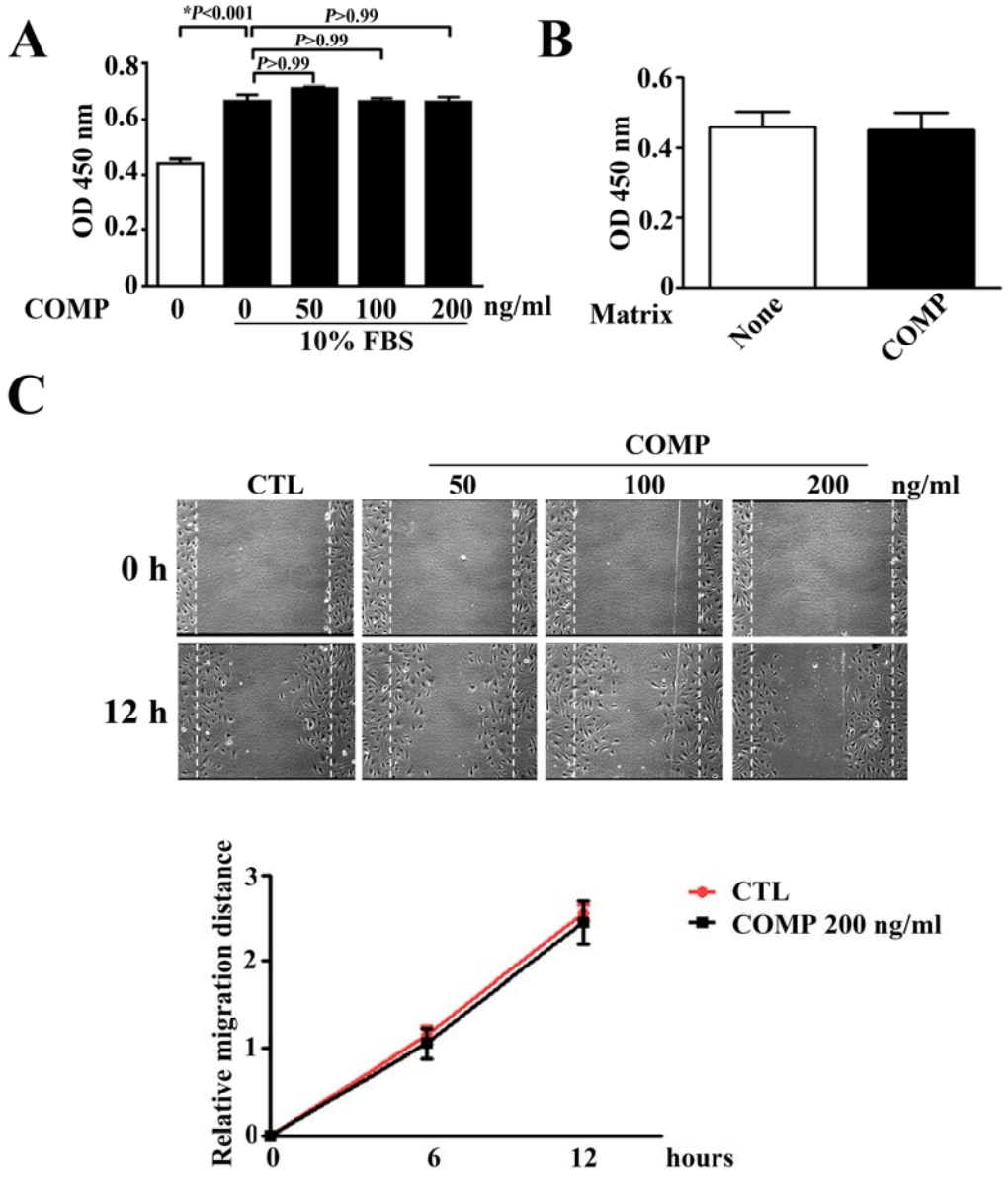



# Supplemental Figure 1

**Supplemental Figure 1. Characterization of ADAMTS-7 neutralization antibody.**

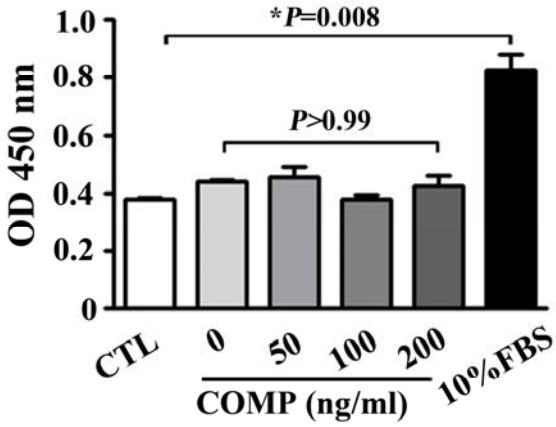
(A) Representative Western Blot analysis of COMP cleavage. COMP-stable transfected 293A cell were infected by Ad-ADAMTS-7, followed by increasing amount of ADAMTS-7

neutralization antibody. Full-length COMP band (110 KD under reducing condition) and a COMP fragment (≈55 KD) were detected. (B) Representative images of HA VSMC migration after scratch would injury in the presence or absence of ADAMTS-7 neutralization antibody. The mean distance migrated by VSMCs was quantified. Results are means±SEM from 3 independent experiments, **P*<0.05. Magnification is ×100.




# Supplemental Figure 2

**D**

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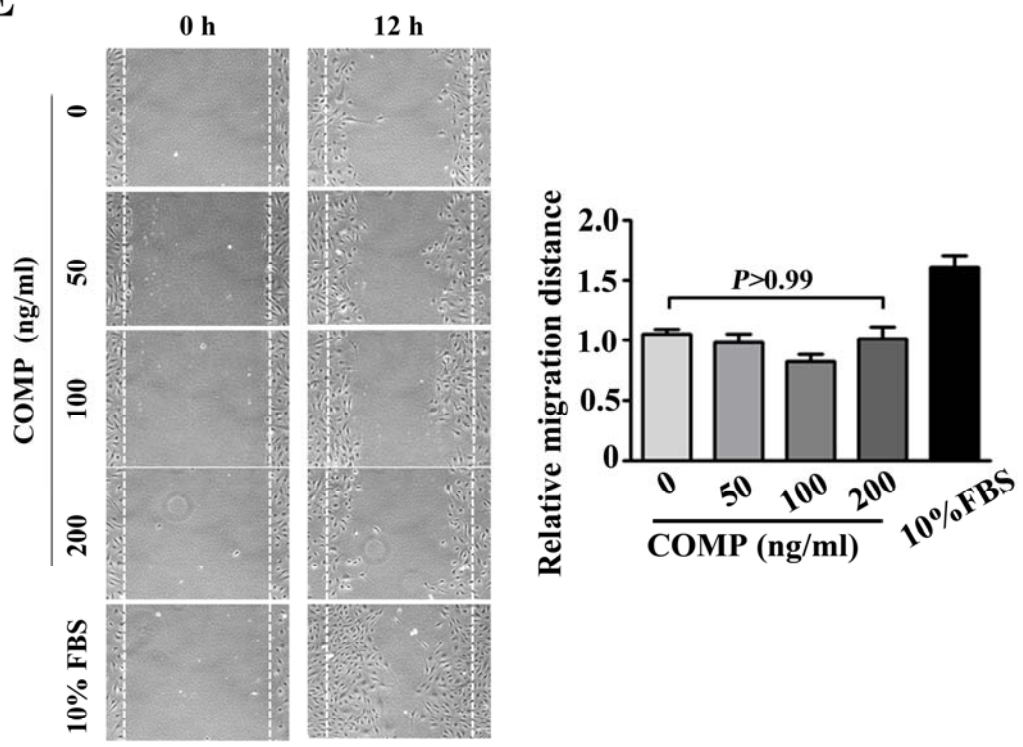
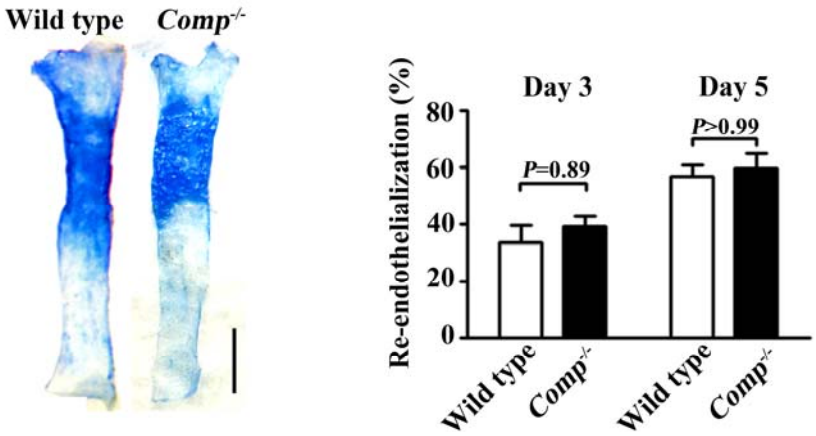
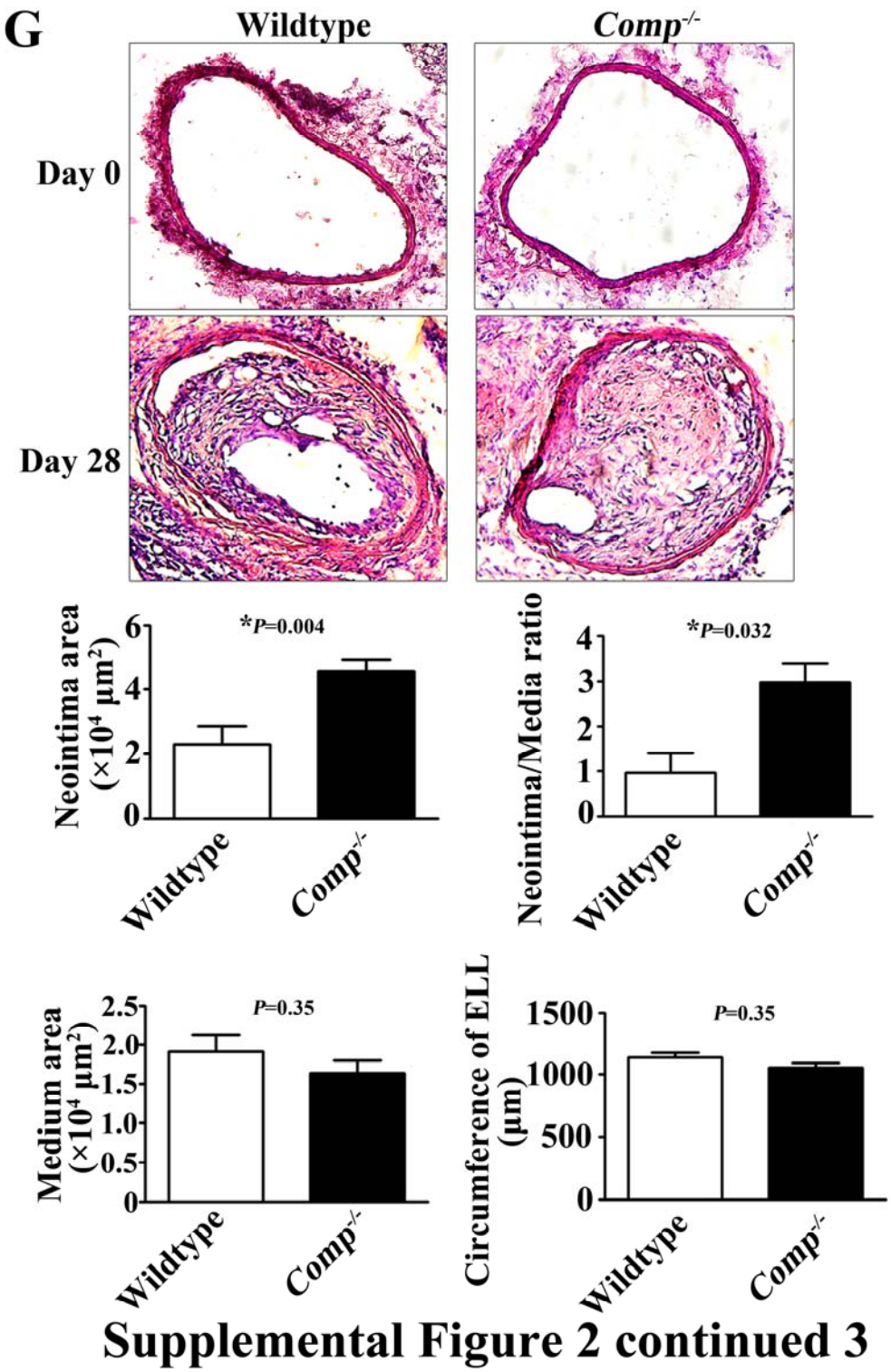



**Supplemental Figure 2 continued**

**F**




# Supplemental Figure 2 continued 2

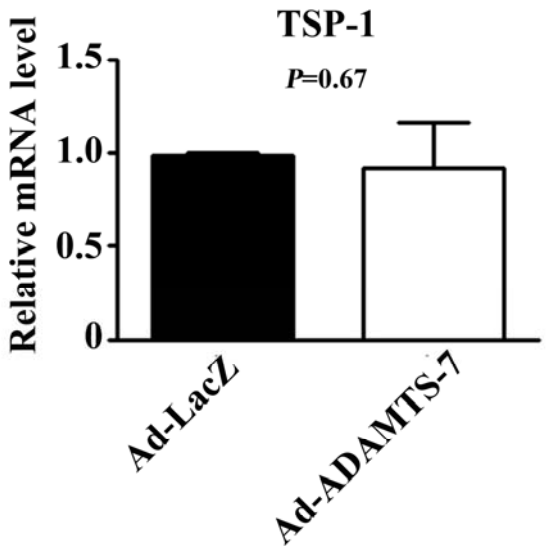



**Supplemental Figure 2 continued 3**

**Supplemental Figure 2. Effects of COMP on HUVEC proliferation and migration.**

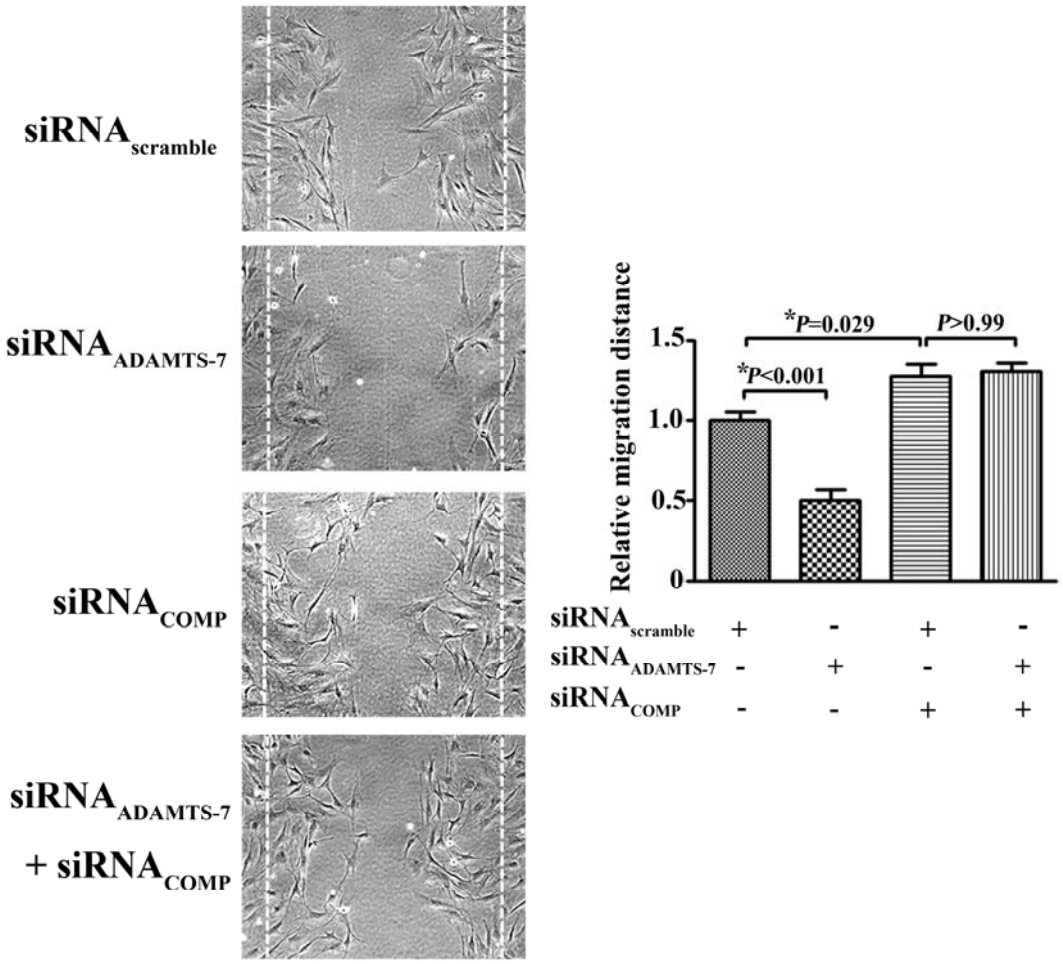
(A) Proliferation of HUVECs with COMP supplement in culture medium via cell counting kit-8. Results are means±SEM from 3 independent experiments performed in duplicate. (B)

HUVECs were cultured on a plate coated with or without COMP protein (30 μg/ml). Cell proliferation was analyzed 24 hours after synchronization. Results are means±SEM from 3 independent experiments. (C) Representative images of cell migration after scratch in COMP-treated HUVECs. The mean distance was quantified. Results are means±SEM from 3 independent experiments. Magnification was ×100. (D&E) Proliferation and migration of HUVECs with COMP under 1% FBS. 10% FBS was a positive control. Results were means±SEM from 3 independent experiments, **P*<0.05. (F) Representative pictures of Evans blue stained carotid arteries 3 or 5 days after vascular injury. Scar bar, 1 mm. n=6-8 for each group. (G) Neointima formation was determined on hematoxylin and eosin–stained cross sections of carotid arteries 0 day and 28 days after vascular wire injury in wild type and *Comp*$^{-/-}$ mice (n=6 each group). Scar bar, 100 μm. **P*<0.05.

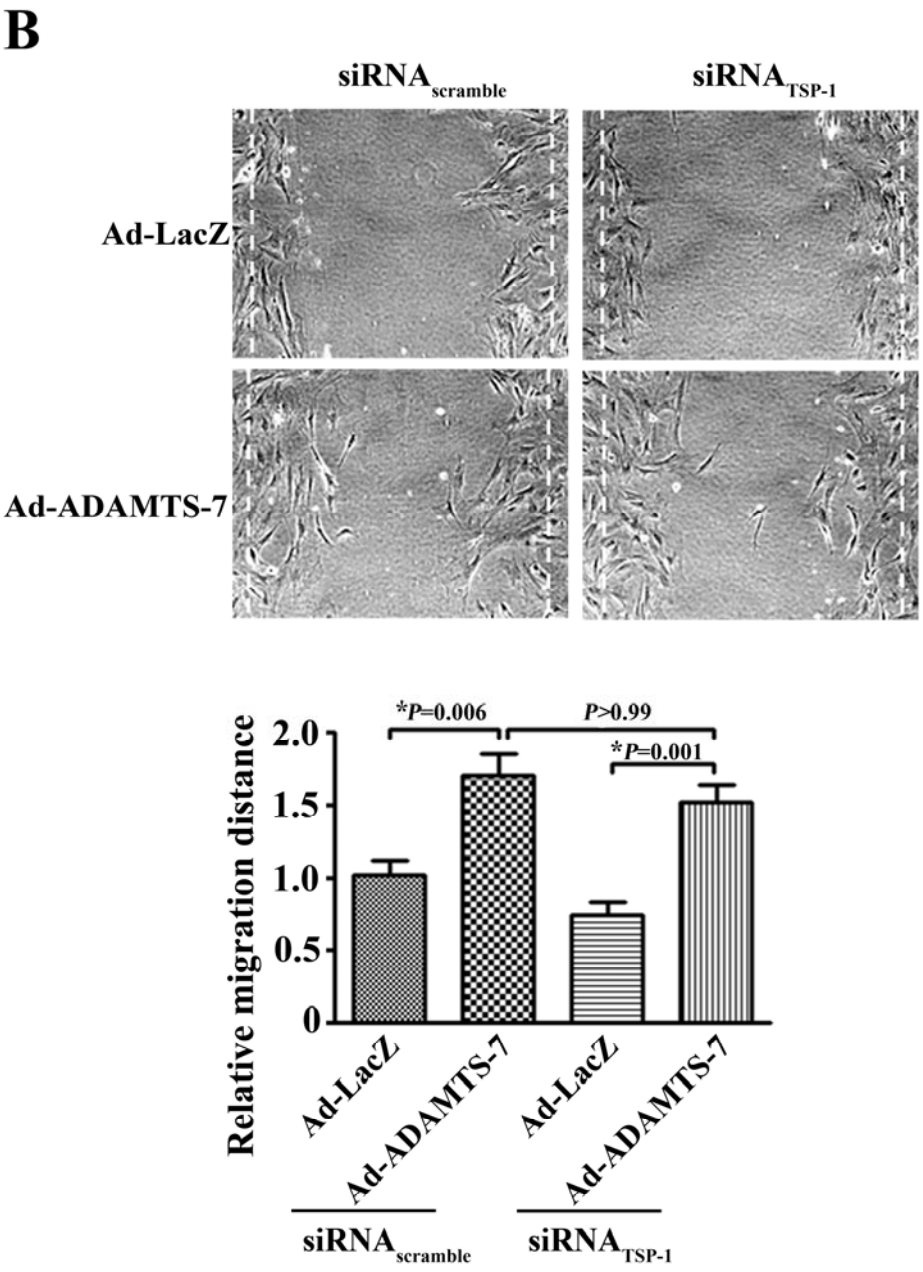



# Supplemental Figure 3

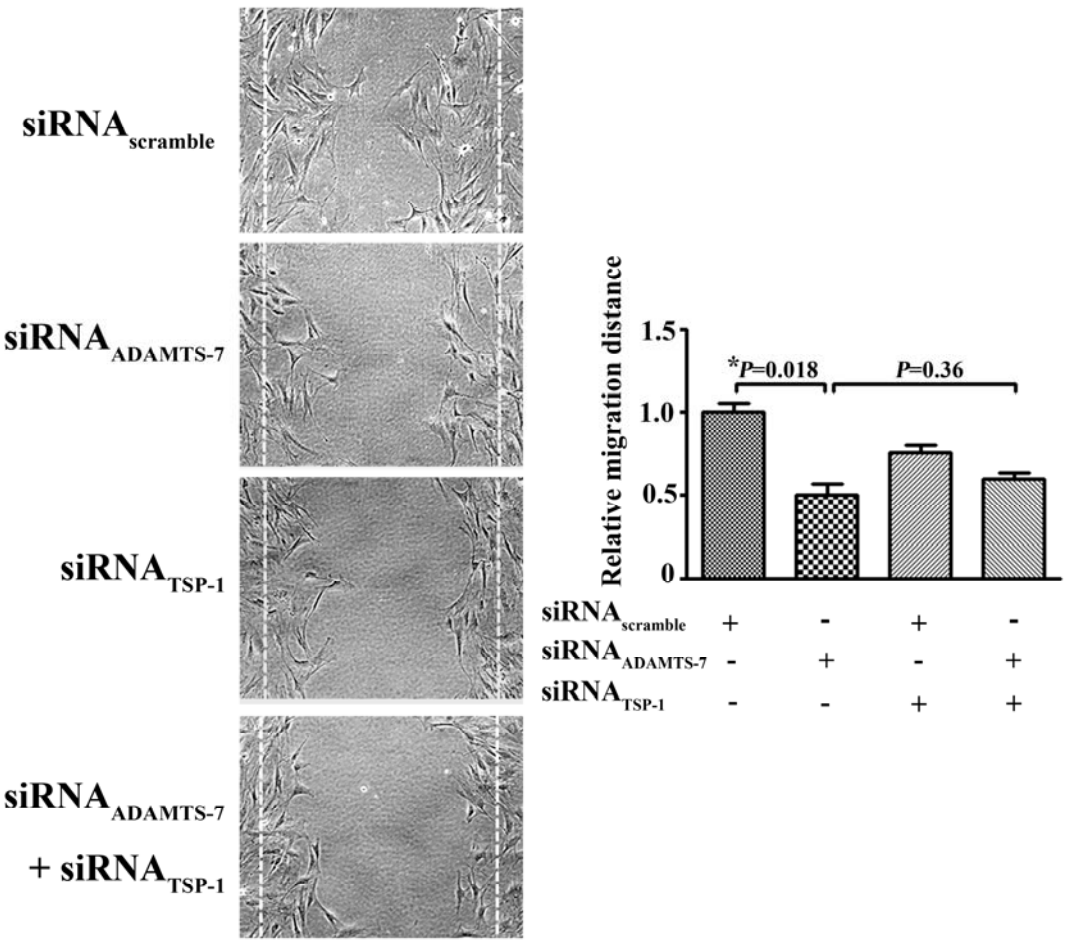
**Supplemental Figure 3. mRNA level of TSP-1 in Ad-*ADAMTS-7*-infected or Ad-*LacZ*-infected HUVECs.** Results are means±SEM from 5 independent experiments.




# Supplemental Figure 4



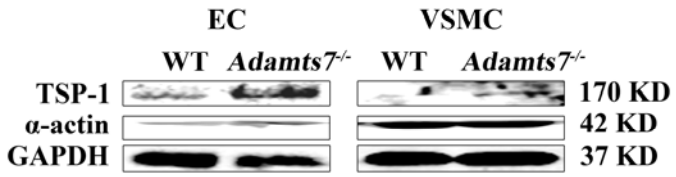

**Supplemental Figure 4 continued**




# Supplemental Figure 4 continued 2

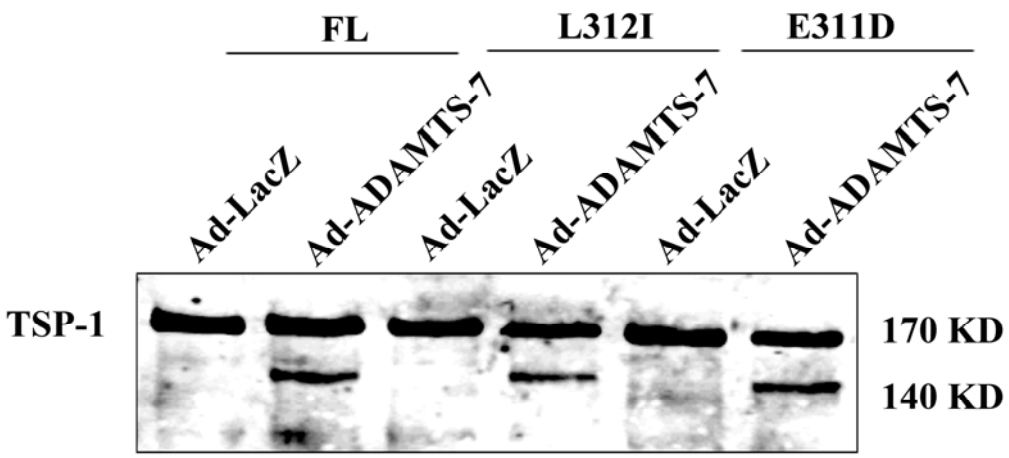
**Supplemental Figure 4. ADAMTS-7 mediates VSMCs migration via COMP but not TSP-1.** (A) Representative images of cell migration 12 hours after scratch in specific siRNA-treated rat VSMCs. Magnification was ×100. (B&C) Effect of ADAMTS-7 overexpression/silencing on cell migration in TSP-1 siRNA-treated rat VSMCs.

Magnification was ×100. The mean distance was quantified. Results are means±SEM from 3 independent experiments, *$P$<0.05.




# Supplemental Figure 5

**Supplemental Figure 5. Protein expression of TSP-1 in EC and VSMC from wildtype (WT) and *Adamts7*$^{-/-}$ aorta respectively.**




# Supplemental Figure 6

**Supplemental Figure 6. Western blot of TSP-1 cleaved by ADAMTS-7, mutant ADAMTS-7 L312I and E311D.**

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**APPENDIX X:**

**[§]German Mouse Clinic, Helmholtz Zentrum München, German Research Center for Environmental Health GmbH, Neuherberg, Germany**

Thure Adler [1,2]

Dirk H. Busch [2]

Antonio Aguilar-Pimentel[1,3]

Markus Ollert[3, 11]

Oana Amarie[1,4]

Tobias Stoeger[1,4]

Ali Önder Yildrim[1,4]

Oliver Eickelberg[4]

Cornelia Prehn[1]

Jerzy Adamski[1]

Lore Becker[1,5]

Alexandra Vernaleken[1, 5]

Thomas Klopstock [5,16,20,21]

Marion Horsch[1]

Johannes Beckers[1,18,19]

Kristin Moreth[1]

Raffi Bekeredjian[6]

Hugo Katus[6]

Lillian Garrett[1,8]

Sabine M. Hölter [1,8]

Annemarie Zimprich[1,8]

Wolfgang Wurst [8,14,15,16,17,21]

Oliver Puk[1,8]

Jochen Graw [8]

Wolfgang Hans[1]

Jan Rozman [1,19]

Martin Klingenspor[9,10]

Laura Brachthäuser[1,7]

Julia Calzada-Wack[1,7]

Dirk Janik[1,7]

Tanja Klein-Rodewald[1,7]

Frauke Neff[1,7]

Ildikó Rácz[1,12]

Andreas Zimmer[12]

Birgit Rathkolb [1,13,19]

Eckhard Wolf [13]

Manuela Gegenfurtner[1]

Ralph Steinkamp[1]

Christoph Lengger[1]

Holger Maier[1]

Claudia Stoeger[1]

Stefanie Leuchtenberger[1]

Valérie Gailus-Durner [1]

Helmut Fuchs [1]

Martin Hrabě de Angelis [1,18,19]

1 German Mouse Clinic, Institute of Experimental Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health GmbH, Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany

2 Institute for Medical Microbiology, Immunology and Hygiene, Technical University of Munich, Trogerstrasse 9, 81675 Munich, Germany

3 Department of Dermatology and Allergy, Biederstein, Klinikum rechts der Isar, Technische Universität München (TUM), Biedersteiner Str. 29, 80802 Munich,

4 Comprehensive Pneumology Center, Institute of Lung Biology and Disease, Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Ingolstädter Landstraße 1, 85764 Neuherberg, Germany and Member of the German Center for Lung Research

5 Department of Neurology, Friedrich-Baur-Institut, Ludwig-Maximilians-Universität München, Ziemssenstrasse 1a, 80336 Munich, Germany

6 Department of Cardiology, University of Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany

7 Institute of Pathology, Helmholtz Zentrum München, German Research Center for

Environmental Health GmbH, Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany

8 Institute of Developmental Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health GmbH, Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany

9 Chair for Molecular Nutritional Medicine, Technische Universität München, Else Kröner-Fresenius Center for Nutritional Medicine, 85350 Freising, Germany

10 ZIEL – Center for Nutrition and Food Sciences, Technische Universität München, 85350 Freising, Germany

11 Clinical Research Group Molecular Allergology, Center of Allergy and Environment Munich (ZAUM), Technische Universität München (TUM), and Institute for Allergy Research, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany

12 Institute of Molecular Psychiatry, University of Bonn, Sigmund-Freud-Strasse 25, 53127 Bonn, Germany

13 Ludwig-Maximilians-Universität München, Gene Center, Institute of Molecular Animal Breeding and Biotechnology, Feodor-Lynen Strasse 25, 81377 Munich, Germany

14 Chair of Developmental Genetics, Center of Life and Food Sciences Weihenstephan, Technische Universität München, Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany

15 Max Planck Institute of Psychiatry, Kraepelinstr. 2-10 , 80804 Munich, Germany

16 Deutsches Institut für Neurodegenerative Erkrankungen (DZNE) Site Munich, Schillerstrasse 44, 80336 Munich, Germany

17 Munich Cluster for Systems Neurology (SyNergy), Adolf-Butenandt-Institut, Ludwig-Maximilians-Universität München, Schillerstrasse 44, 80336 Munich, Germany

18 Chair of Experimental Genetics, Center of Life and Food Sciences Weihenstephan, Technische Universität München, Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany

19 Member of German Center for Diabetes Research (DZD), Ingolstaedter Landstraße 1, 85764 Neuherberg, Germany

20 German Network for Mitochondrial Disorders (mitoNET)

21 German Center for Vertigo and Balance Disorders, Munich, Germany