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# DOCK2 deficiency attenuates abdominal aortic aneurysm formation

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## Abstract

**Background:** Abdominal aortic aneurysm (AAA) is a potentially lethal disease that lacks pharmacological treatment. Degradation of extracellular matrix proteins especially elastin laminae is the hallmark for AAA development. Dedicator of cytokinesis 2 (DOCK2) has shown proinflammatory effects in several inflammatory diseases and acts as a novel mediator for vascular remodeling. However, the role of DOCK2 in AAA formation remains unknown.

**Methods:** Angiotensin II (Ang II) infusion of apolipoprotein E deficient (ApoE−/−) mouse and topical elastase-induced AAA combined with DOCK2 knockout (DOCK2−/−) mouse models were used to study DOCK2 function in AAA formation/dissection. The relevance of DOCK2 to human AAA was examined using human aneurysm specimens. Elastin fragmentation in AAA lesion was observed by elastin staining. Elastin-degrading enzyme matrix metalloproteinase (MMP) activity was measured by in situ zymography.

**Results:** DOCK2 was robustly up-regulated in AAA lesion of angiotensin II (Ang II)-infused ApoE−/− mice, elastase-treated mice as well as human AAA lesions. Knockout of DOCK2 (DOCK2−/−) significantly attenuated the Ang II-induced AAA formation/dissection or rupture in mice along with reduction of monocyte chemoattractant protein-1 (MCP-1) and matrix

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Supplemental Materials:

Expanded Materials & Methods

Online Figure S1–S3

Online Table S1–S3

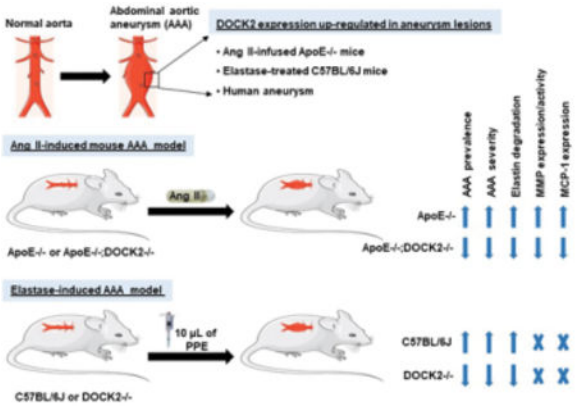
Major Resources Table

**Disclosures:** None.

metalloproteinase (MMP) expression and activity. Accordingly, the elastin fragmentation observed in ApoE−/− mouse aorta infused with Ang II and elastase-treated aorta was significantly attenuated by DOCK2 deficiency. Moreover, DOCK2−/− decreased the prevalence and severity of aneurysm formation as well as the elastin degradation observed in the topic elastase model.

**Conclusion:** Our results indicate that DOCK2 is a novel regulator for AAA formation. DOCK2 regulates AAA development by promoting MCP-1 and MMP2 expression to incite vascular inflammation and elastin degradation.

## Graphical Abstract




### Keywords

Dedicator of cytokinesis 2; Angiotensin II; Abdominal aortic aneurysm; MMP

## Introduction

Abdominal aortic aneurysm (AAA) is a focal dilatation of the abdominal aorta with an aortic diameter over 3 cm (normally 2 cm), or more than 50% of normal diameter[1–3]. The pathogenesis of AAA is a complex process involving several key events including chronic vascular inflammation, excessive local production of matrix-degrading proteases, and fragmentation of elastin laminae in aortic wall[3–5]. AAA is a potentially lethal disease with a mortality of 90% when it ruptures[3,6]. Currently, the therapy for AAA mainly depends on surgery such as endovascular stent graft repair[3,7]. There is a lack of pharmacological treatment for AAA. β-blocker has been frequently used for patients with small AAA that does not reach the size of surgery criteria, but it has severe side effects such as bradycardia[8–10]. Therefore, it is crucial to identify key regulators promoting the pathogenesis of AAA for developing effective therapeutics.

Dedicator of cytokinesis 2 (DOCK2) is initially found to be expressed in lymphocytes and macrophages of various organs, and play important roles in neutrophil chemotaxis, lymphocyte activation, and dendritic cell type I interferon induction[11,12]. DOCK2 deficiency enables long-term cardiac allograft survival[13]. We have reported that DOCK2 deficiency protects the mice from HFD-induced obesity by attenuating adipose tissue and system inflammation and by increasing energy expenditure in adipose tissue[14]. In addition, we

have identified DOCK2 as a novel regulator of vascular remodeling in injured arteries, i.e., DOCK2 deficiency blocks injury-induced intimal hyperplasia[15,16]. Since inflammation and vascular remodeling are key pathological processes in AAA development, we hypothesized that DOCK2 may be involved in AAA formation.

In the present study, we found that DOCK2 expression was dramatically upregulated in the aneurysm lesions in both mouse AAA models and human AAA patients. DOCK2 knockout (DOCK2−/−) significantly decreased the prevalence and severity of AAA/dissection induced by either angiotensin (Ang) II infusion or topic application of elastase in mouse infrarenal aorta. DOCK2 appears to promote AAA formation by causing aorta wall elastin degeneration via enhancing MCP-1 and MMP production/activity. Our study represents the first demonstration that DOCK2 is novel regulator essential for AAA development.

## Methods

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Please see the Major Resources Table in the Supplemental Materials.

### Animal procedures:

All animals were housed under conventional conditions in the animal care facilities and received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals. Animal experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Missouri. C57BL/6J mice were purchased from the Jackson Laboratory. DOCK2−/− mice were generated as previously described[17] and have been bred with C57BL/6J for more than 10 generation. DOCK2−/− mice were then crossbred with ApoE−/− mice (The Jackson Laboratory) to produce ApoE−/−; DOCK2−/− mice. ApoE−/− mice serve as controls for the study. The mice in different groups were not from the same parents, but they were all in C57BL/6J genetic background. In addition, sex- and age-matched animals were used for each study. Therefore, the genetic variations among groups are likely to be minimal and not affect the conclusions. The number of animals was determined by power calculation based on the prior experience to detect a 30% reduction in AAA prevalence with DOCK2 deficiency. Mice were provided regular drinking water and standard chow diet (4% fat diet, #7001) from Harlan-Teklad (Madison, Wisconsin).

### Ang II infusion-induced mouse AAA model:

The mouse model of Ang II-induced AAA formation has been previously described[18–20]. Male, hyperlipidemic ApoE−/− and ApoE−/−; DOCK2−/− mice at 10 to 12 weeks of age were used for assessing AAA incidence and severity. The mice were infused with saline or Ang II via subcutaneous osmotic minipumps (Alzet, model 2004, Palo Alto, CA) at 1,000 ng/kg/min for 14 or 28 days. The abdominal aortas were harvested after perfusion with PBS under a pressure lower than the physiological blood pressure, which was achieved by gently pushing a 3ml syringe. AAA was defined by the increase of maximal aortic diameter

by 50% or greater compared to the mean maximal aortic diameter of saline-infused mice. The measurement of the external aorta diameter was done in situ at its maximal width for each mouse. AAA severity was determined using a classification scheme described previously[20,21].

### Topical elastase-induced AAA model:

The elastase-induced AAA mouse model was established as described previously[22]. Briefly, 8–10 week old male C57BL/6J or DOCK2−/− mice were anesthetized with 2% Isoflurane, placed on a heating pad, and received subcutaneous injection of buprenorphine (0.1 mg/kg). A medial laparotomy was performed, and the infrarenal aorta from the left renal vein to the iliac bifurcation was isolated and bathed in either 10 μL of filtered porcine pancreatic elastase (PPE, E1250, Sigma-Aldrich) or heat-inactivated elastase (control) for 10 min. The external aortic diameters were measured in situ before elastase application and at 14 days after elastase treatment. The aorta lumen dilation was observed, and the diameters measured by using Vevo 1100 imaging system with B mode (FUJIFILM VisualSonics).

### Human AAA specimens:

Human healthy abdominal aorta and AAA specimens were obtained from Mizzou OneHealth Biorepository. The patient information was de-identified and is included in Table S1. Use of human specimens was approved by the Institutional Review Board of University of Missouri (IRB # 2026026).

### Western blotting (WB) analysis:

Protein extraction and Western blotting were performed as described previously[23,24]. The protein concentration was determined by BCA Protein Assay Reagent (ThermoFisher Scientific #23227). Equal amounts of proteins (30 μg) calculated based on the protein concentration for each sample were loaded for the detection of each protein of interest including the internal control (GAPDH). The proteins were resolved in 4–20% SDS polyacrylamide gel and transferred to nitrocellulose membranes. Antibodies against DOCK2 (Millipore Sigma, Cat # 09–454), MCP-1 (Abcam, Cat# ab25124), MMP2 (Abcam, Cat# ab86607), and GAPDH (Proteintech, Cat # 1E6D9) were used for immunoblotting. Protein expression was detected using an enhanced chemiluminescence kit (Millipore).

### Histology and immunohistochemistry (IHC) staining:

Mouse abdominal aortas were fixed in 4% PFA, dehydrated, and embedded in paraffin. Sections (5 μm) were cut, deparaffinized, and stained with hematoxylin and eosin (H&E) for structural observation and Van Gieson stain reagents for elastin. Images were captured using a Nikon microscope. Pathological score about elastin degradation in the aortas was assessed blindly from the treatment and genotyping according to the following rules: score 1 for degradation less than 25%; score 2 for degradation ranging from 25% to 50%; score 3 for degradation ranging from 50% to 75%; score 4 for degradation greater than 75%[25]. Ten discontinuous sections in the middle part of each specimen with 20 μm apart (every 5$^{th}$ section) were used to quantify elastin degradation, and average score were the mean value of all specimens in each group.

The expression of DOCK2 in tissue sections were assessed by IHC staining as described previously[23,26]. Briefly, sections were rehydrated and blocked with 5% goat serum for 30 min, incubated with primary antibodies against DOCK2 or rabbit normal IgG (negative control) overnight at 4°C followed by incubation with horseradish peroxidase-conjugated secondary antibody. 3,3'-Diaminobenzidine was used to detect the DOCK2 staining, and the sections were counterstained with hematoxylin. Images were captured with a Nikon microscope (Nikon America, Inc.)

### Immunofluorescent staining:

For fluorescent staining, sections were rehydrated and immunostaining performed as described previously[24,27]. After 3 times of washing with phosphate-buffered saline (PBS), the sections were permeabilized with 0.01% Triton X-100 in PBS for 15 min, blocked with 5% goat serum for 30 min, and then incubated with DOCK2 antibody or rabbit normal IgG (negative control) followed by incubation with FITC-conjugated secondary antibodies. Cell nuclei were stained with DAPI (Vector Laboratories, Inc). Stained tissue sections were imaged using a Nikon fluorescent microscope.

### In situ zymography:

In situ zymography was performed using EnzChek® gelatinase/collagenase assay kit (Thermo Fisher Scientific Inc.) to detect MMP activity in abdominal aorta. The experiment was revised based on manufacturer's instruction. Aorta cryosections (10 μm) were air-dried for 5 min in room temperature then washed with PBS twice. Sections were incubated with a fluorogenic gelatin substrate (DQ gelatin) for 2 h at 37° C in a humidified chamber and protected from light. After incubation, the sections were washed twice in PBS and once in distill $H_2O$. Proteolytic activity was detected as green fluorescence signal. For negative controls, sections were incubated with 1,10-phenanthroline for 1 h before adding substrate, and no detectable gelatinolytic activity was observed. Cell nuclei were stained with DAPI. Stained tissue sections were imaged using a Nikon fluorescent microscope. The MMP activity was measured by the fluorescent signal intensity less the negative control, and relative MMP activity was calculated by normalizing to the fluorescent signal intensity in WT aorta infused with saline, which was set as 1.

### Statistical analysis:

Sample size estimation was based on previous results in comparable studies, assuming 80% power at a significance level of 0.05. Specific details on independent biological repeats or animal numbers were provided in corresponding figure legends. Data represent independent biological repeats but not technical replicates and were shown as the mean ± SD. Normality of data was assessed by the D'Agostino & Pearson normality test with alpha=0.05 (Table S2). For comparisons of two groups, since all data were not normally distributed, Mann-Whitney two tailed test was used. For more than 2 groups, 2-way ANOVA with Tukey post-test analysis was used for normally distributed data and Kruskal-Wallis test with Dunn's multiple comparisons test was used for non-normally distributed data. For categorical data (i.e., AAA prevalence), Fisher exact test was used. All p-values and corresponding statistical test was provided (Table S3). Prism 9.0 (GraphPad Software, CA)

or RStudio (Desktop 1.4.1717) was used for statistical analyses, and differences considered statistically significant when nominal P<0.05 or adjusted P<0.05 in case of multiple testing.

## Results:

### DOCK2 is induced in mouse and human AAA and essential for the AAA development in Ang II infusion model.

Since DOCK2 plays essential roles in both inflammatory cells and SMC phenotype as well as vascular remodeling, we hypothesized that DOCK2 is involved in AAA pathogenesis. To test the hypothesis, we first examined DOCK2 expression in AAA tissues in Ang II infusion-induced AAA mouse model[18]. Immunohistochemistry staining showed that although DOCK2 was barely detectable in normal healthy mouse abdominal aorta, it was significantly induced in AAA tissues (Figure 1, A-B). DOCK2 was shown to be expressed in both the adventitia and media areas of the AAA lesion where the pathogenesis of AAA was manifested (Figure 1B). Quantitative analyses showed that DOCK2 was significantly elevated in the AAA lesions (Figure 1C). To determine if DOCK2 is relevant to human AAA development, we examined DOCK2 expression in human AAA lesion. Although DOCK2 was undetectable in healthy human abdominal aorta, it was significantly upregulated in AAA lesion of human patients (Figure 1, D–E), suggesting that DOCK2 may also be involved in AAA pathogenesis in human patients.

To determine if DOCK2 is essential for AAA development, we crossbred DOCK2−/− mice with ApoE−/− mice (WT) to generate ApoE−/−; DOCK2−/− (DOCK2−/−) mice. Only male mice were included in this study following the ATVB Council recommendation for AAA studies that identifying mechanisms of reduced AAA formation focus on males due to a higher AAA prevalence in males[28]. We infused 10- to 12-week-old WT and DOCK2−/− mice with either Ang II (1,000 ng/kg/min) or saline via subcutaneous osmotic minipumps for 28 days and recorded AAA/dissection prevalence and severity. While the Ang II-infused WT mice readily developed AAA/dissection with a prevalence of 75% (9/12 vs 0/12 for saline-infused mice; P=0.001, Fisher Exact test) and exhibited enlarged external aortic diameters, AAA/dissection prevalence was significantly reduced in Ang II-infused DOCK2−/− mice (1/12 vs 9/12 for Ang II-infused WT mice; P=0.024). The representative images were shown in Figure 1F. The DOCK2−/− mice also had less-severe aortic pathology, manifested by a lower mean external aortic diameter (Figure 1G). These data indicated that DOCK2 is a novel protein factor important for the development of AAA/dissection.

### DOCK2 promotes AAA formation by causing elastin breakage via increasing MMP production/activity.

Histological analyses showed that Ang II infusion caused aorta lumen expansion, media degeneration, thrombus formation indicating a dissection or contained rupture (Figure S1) as well as elastin breakage in WT mice (Figure 2A). However, elastin fragmentation was attenuated in DOCK2−/− mouse aorta with Ang II infusion (Figure 2A and Figure S1). Consequently, DOCK2 deficiency significantly reduced the elastin degradation score for aortas infused with Ang II (Figure 2B). Since elastin fragmentation was caused largely by

matrix metalloproteinases (MMP), we measured the MMP production and the expression of inflammation marker monocyte chemoattractant protein-1 (MCP-1) in these aortas. We measured MCP-1 because blocking MCP-1 signaling either through genetic deletion or siRNA-mediated knockdown, or through inhibition of its receptor, CCR2, successfully inhibits AAA development[29–31]. As shown in Figure 2, C-E, Ang II infusion significantly elevated the expression of MCP-1 and MMP2. However, DOCK2−/− attenuated the MCP-1 and MMP2 expression (Figure 2, C-E). To test if MMP activity was altered in the aortas of the WT and DOCK2−/− mice, we performed In Situ Zymography in aortas of mice infused with saline or Ang II for 14 days. As shown in Figure 2, F-G, DOCK2−/− significantly reduced the MMP activities observed in Ang II-infused WT mouse aortas. These data suggested that DOCK2 promotes AAA development by increasing MMP production and activity.

### DOCK2 promotes AAA formation in topical elastase model.

In order to further determine the role of DOCK2 in AAA development, a second AAA model, i.e., topical elastase model, was performed in mouse infrarenal aortas. Elastase application caused aorta dilation as shown by both lumen and external diameter enlargement, inflammatory cell infiltration, and adventitia thickening (Figure S2–3), which are the typical pathological features observed in human AAA. Importantly, DOCK2 was highly upregulated in the AAA lesion. It appears that DOCK2 was expressed in multiple cells in the media and adventitia layers (Figure S2B). DOCK2 deficiency significantly blocked the elastin degradation caused by elastase incubation (Figure S3A). Consequently, DOCK2 deficiency decreased the AAA prevalence from 75% (6/8) to 37.5% (3/8) in elastase-treated aortas. DOCK2 deletion also markedly reduced elastin degradation score and aorta external and lumen diameters in mouse aortas treated with elastase (Figure S3, B–E). These data indicated that DOCK2 contributes to AAA formation in different models.

## Discussion

This study reveals the first causal link between DOCK2 and AAA formation. The dramatic reduction of the aneurysm/dissection prevalence and severity as well as aortic dilation (in elastase model) and elastin fragmentation in DOCK2−/− mouse aorta establishes DOCK2 as a novel regulator for AAA/dissection. Our study also indicates that DOCK2 upregulation leads to the elastin degeneration, at least in an indirect manner, and further aneurysm formation by increasing MCP-1 expression and MMP production as well as enhancing MMP activation as shown by the reduced proteolytic activity.

DOCK2 function in AAA was determined by using two AAA models, i.e., the Ang II-infused ApoE−/− mouse model and peri-adventitial elastase application model[22]. The Ang II infusion model shares several common features with human AAAs such as elastic media degeneration, aortic dissection, aneurysmal wall remodeling, macrophage infiltration, thrombus formation, and aortic rupture[32]. However, Ang II-infusion-induced aneurysms occur most frequently in the suprarenal aorta as opposed to the infrarenal location observed in humans. Peri-adventitial elastase model mimics human AAA where aneurysm forms in infrarenal aorta with degradation of elastic lamina, increased macrophage infiltration,

reduced SMC protein expression, and increased MMP activity[33]. In addition, the fusiform aneurysms formed by the peri-adventitial application model are more consistent with those seen in human AAAs[33]. The combined use of the Ang II-infusion and PAE models demonstrate that DOCK2 plays a critical role in AAA formation and make our studies more relevant to the human disease. Indeed, DOCK2 is highly induced in human AAA lesion.

AAA development involves multiple pathological processes in which multiple cells such as macrophages, T cells, smooth muscles, adventitia fibroblasts play different roles. DOCK2 appears to be induced in multiple cells in the AAA lesions, suggesting that DOCK2 is involved in multiple cellular functions important for AAA formation. Although our current study is limited to the global determination of DOCK2 function without specifying its cell-specific roles and the underlying cellular or molecular mechanisms in AAA development, our findings demonstrate that DOCK2 is a novel factor critical for AAA formation. How DOCK2 regulates functions of different cells such as smooth muscles and macrophages to control AAA development would require extensive future studies using tissue-specific knockout mice. The outcome of these studies would eventually establish DOCK2 as a therapeutic target for AAA treatment in the future. As a matter of fact, since DOCK2 is an activator of Rac1 activity[17], and inhibiting Rac1 activity seems to reduce AAA progression[34], blocking DOCK2 expression or activity is likely to attenuate AAA formation, which would also be an important subject for the future study.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Sources of Funding:

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## Nonstandard Abbreviations and Acronyms

| | |
|---|---|
| **DOCK2** | Dedicator of cytokinesis 2 |
| **Ang II** | Angiotensin II |
| **SMC** | Smooth muscle cell |
| **AAA** | Abdominal aortic aneurysm |
| **MMP** | Matrix metalloproteinases |
| **ApoE−/−** | Apolipoprotein E deficient |
| **MCP-1** | Monocyte Chemoattractant Protein-1 |

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**Highlights:**

- DOCK2 is upregulated in both mouse and human AAA lesions.
- DOCK2 deficiency in mice significantly blocks AAA formation in both Angiotensin II infusion and peri-adventitial elastase models.
- DOCK2 promotes AAA/dissection likely by altering MMP production and activation, which degrades elastin laminae and thus causes aorta dilation and media dissection or rupture.

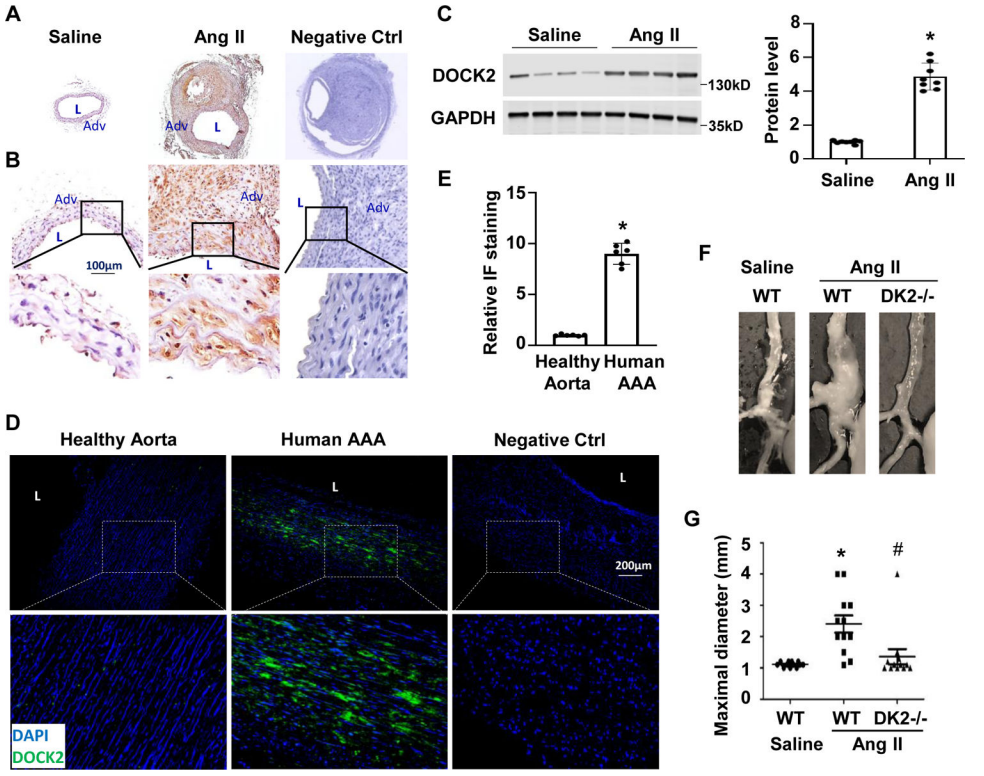


**Figure 1. DOCK2 is essential for abdominal aortic aneurysm (AAA) formation.**

ApoE−/− mice were infused with saline or angiotensin II (Ang II, 1000 ng/kg/min) for 28 days. **A-C**, DOCK2 is induced in AAA lesions of ApoE−/− mice infused with angiotensin II (1000 ng/kg/min) as shown by immunohistochemistry staining (A-B) and Western blotting with DOCK2 antibody (C). Images with lower magnification in **A** show the aneurysm formation/dissection/rupture. DOCK2 is expressed in both the medial and adventitial layers. GAPDH is the internal control. *P<0.001 vs. saline-infused group, n=8 mice per group. **D**, DOCK2 is upregulated in human AAA lesions, but not in healthy aortas, as detected by immunofluorescent staining. DAPI stains nuclei. Scale Bar: 200um. **E**, The DOCK2 levels were quantified by normalizing the staining intensity to the staining in healthy human abdominal aorta. *P=0.0022 vs. healthy aorta, n=6 per group. **F-G**, ApoE−/− (WT) and ApoE−/−; DOCK2−/− (DK2−/−) mice were infused with saline or Ang II (1000 ng/kg/min) for 28 days. Gross abdominal aorta images were shown. DOCK2−/− blocks Ang II-induced AAA/dissection with significant reduction in maximal external aorta diameters (G). *P=0.017 vs. saline-infused WT mice; #P=0.024 vs. Ang II-infused WT mice; n=12 mice per group.

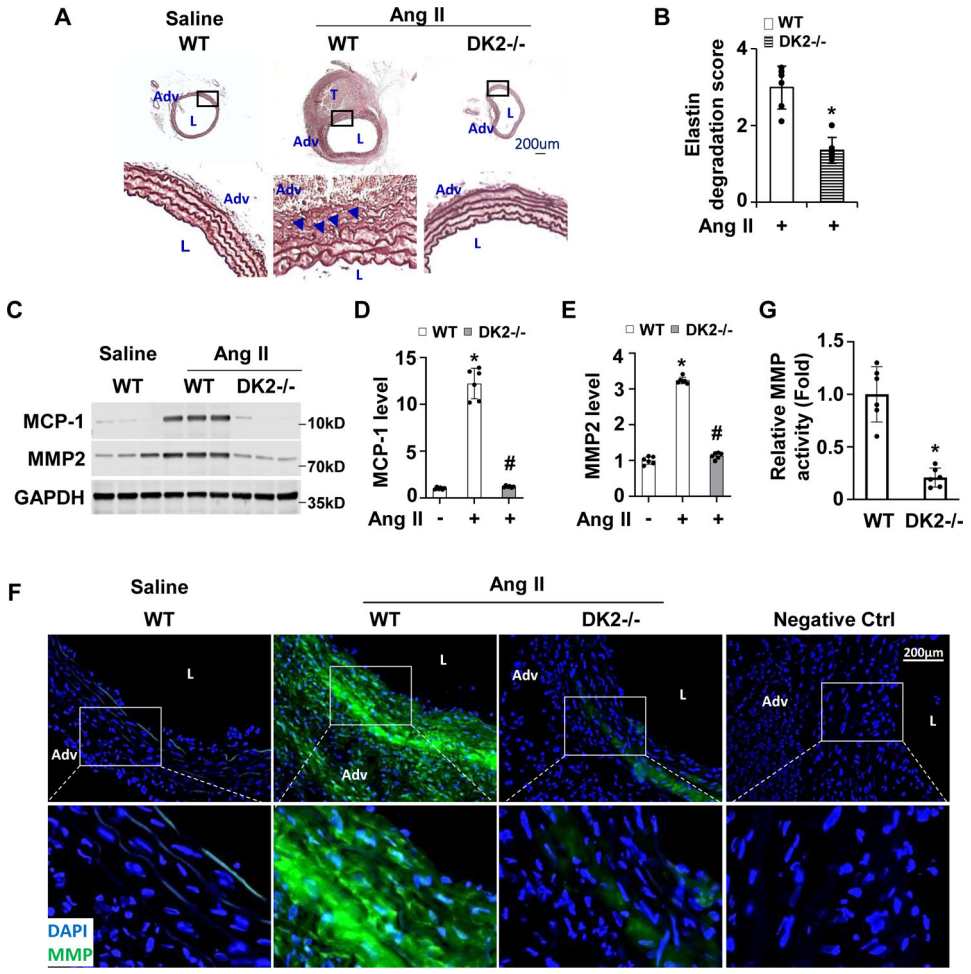


**Figure 2. DOCK2 promotes elastin breakage by mediating MMP production and activities.**

**A-B**, ApoE−/− (WT) and ApoE−/−; DOCK2−/− (DK2−/−) mice were infused with saline or angiotensin II (Ang II, 1000 ng/kg/min) for 28 days. Elastin staining shows aorta lumen expansion, elastin breakage (arrowheads), thrombus formation indicating aorta dissection or rupture in WT, but not in DK2−/−, mice infused with Ang II **(A)**. L: lumen; T: thrombus; Adv: adventitia. The higher magnification images in the lower panel were enlargements of the areas in the rectangle box in the upper panels. Elastin fragmentation was significantly blunted by DK2−/− as compared to the WT mice **(B)**. Five sections in each specimen were used to quantify elastin degradation score, and average scores were obtained from the mean value of all specimens in each group. *P=0.012 vs. WT mice infused with Ang II (n=6 mice per group). **C-E**, Mice were infused with saline or angiotensin II (Ang II, 1000 ng/kg/min) for 14 days. Abdominal aorta proteins were extracted. MCP-1 and MMP2 protein expression in mouse abdominal aorta was detected by Western blot **(C)**. MCP-1 protein levels in C were quantified by normalizing to GAPDH **(D)**. *P<0.001 vs. WT mice with saline infusion; #P<0.001 vs. WT mice with Ang II infusion; n=6 mice per group. MMP2 protein levels in C were quantified by normalizing to GAPDH **(E)**. *P=0.0074 vs. WT mice with saline infusion; #P=0.012 vs. WT mice with Ang II infusion; n=6 mice per group. **F-G**, WT and DK2−/− mice were infused with saline or Ang II (1000 ng/kg/min) for 14 days. MMP

activities were determined by in situ zymography, and fold change was calculated relative to the WT with Ang II group. *P=0.0022 vs. WT mice with Ang II infusion; n=6 mice per group.