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Poldip2 sustains vascular structure and function

Roy L. Sutliff[1], **Lula L. Hilenski**[2], **Angélica M. Amanso**[2], **Ioannis Parastatidis**[2], **Anna E. Dikalova**[2], **Laura Hansen**[3], **Srinivasa Raju Datla**[2], **James S. Long**[2], **Alexander M. El-Ali**[1], **Giji Joseph**[2], **Rudolph L. Gleason Jr.**[3], **W. Robert Taylor**[2,3], **C. Michael Hart**[1], **Kathy K. Griendling**[2], and **Bernard Lassègue**[2,*]

[1]Atlanta Veterans Affairs Medical Center and Emory University School of Medicine, Department of Medicine, Division of Pulmonary and Critical Care Medicine, Decatur, GA 30033

[2]Emory University School of Medicine, Department of Medicine, Division of Cardiology, Atlanta, GA 30322

[3]Emory University School of Medicine and Georgia Institute of Technology, Wallace H. Coulter Department of Biomedical Engineering, Atlanta, GA 30332

Abstract

Objective—Based on previous evidence that polymerase delta interacting protein 2 (Poldip2) increases NADPH oxidase 4 (Nox4) activity in vascular smooth muscle cells (VSMC), we hypothesized that in vivo knockdown of Poldip2 would inhibit reactive oxygen species (ROS) production and alter vascular function.

Approach and Results—Because homozygous Poldip2 deletion is lethal, Poldip2+/− mice were employed. Poldip2 mRNA and protein levels were reduced by about 50% in Poldip2+/− aorta, with no change in p22phox, Nox1, Nox2 and Nox4 mRNAs. NADPH oxidase activity was also inhibited in Poldip2+/− tissue. Isolated aortas from Poldip2+/− mice demonstrated impaired phenylephrine and potassium chloride-induced contractions, increased stiffness and reduced compliance, associated with disruption of elastic lamellae and excessive extracellular matrix deposition. Collagen I secretion was elevated in cultured VSMC from Poldip2+/− mice and restored by H_2O_2 supplementation, suggesting that this novel function of Poldip2 is mediated by reactive oxygen species. Furthermore, Poldip2+/− mice were protected against aortic dilatation in a model of experimental aneurysm, an effect consistent with increased collagen secretion.

Conclusions—Poldip2 knockdown reduces H_2O_2 production in vivo, leading to increases in extracellular matrix, greater vascular stiffness and impaired agonist-mediated contraction. Thus, unaltered expression of Poldip2 is necessary for vascular integrity and function.

Keywords

Poldip2; Extracellular matrix; Hydrogen peroxide; Nox4; Blood vessel

[*]corresponding author: Bernard Lassègue, Emory University, Cardiology, 101 Woodruff Circle, WMB 308-B, Atlanta, GA 30322, phone: 404-727-3138, bernard.lassegue@emory.edu.

Disclosures
None

Introduction

Vascular remodeling occurs in response to elevated blood pressure, vessel injury, endothelial dysfunction, as well as leukocyte infiltration, and is exacerbated in aging. Documented changes in vascular structure in the adult include vascular smooth muscle hypertrophy and hyperplasia, accumulation of extracellular matrix and loss of elasticity. Excess matrix can lead to loss of contractility and arterial stiffening, both of which have important consequences for vascular function. Over the past decade, it has been suggested that aortic stiffness can be considered an important 'tissue biomarker' risk factor for cardiovascular disease.[1] Indeed, a recent meta-analysis of 17 clinical studies showed that aortic stiffness, as measured by pulse wave velocity, is a strong predictor of future cardiovascular events and all-cause mortality.[2] It is therefore of critical importance to understand the mechanisms contributing to the regulation of vascular compliance.

One newly appreciated regulator of aortic stiffness is oxidative stress. Zhou et al.[3] found that aged mice deficient in superoxide dismutase 2 (SOD2) with reduced H_2O_2 exhibit increased pulse wave velocity, increased collagen I expression, impaired integrity of elastic lamellae, and enhanced medial SMC apoptosis. Of interest, a similar phenotype was not observed in mice deficient in p47phox, a component of the Nox1 and Nox2 NADPH oxidases. However, Maiellaro-Rafferty et al.[4] found that ApoE−/− mice crossed with smooth muscle-specific catalase overexpressing mice have increased aortic stiffness and greater collagen content. Other work suggests that reactive oxygen species (ROS) derived from Nox4 NADPH oxidase can also affect extracellular matrix composition and structure. Nox4 increases collagen expression in cardiac myofibroblasts,[5] matrix metalloproteinase-2 (MMP-2) activity in human ovarian cancer cells,[6] activation of MMP-1 by diesel exhaust fumes in lung epithelial cells,[7] and urotensin II-induced activation of MMP-2 in vascular smooth muscle cells (VSMC).[8] Moreover, genetic deletion of Nox4 leads to increased interstitial cardiac fibrosis in response to suprarenal aortic constriction.[9] These observations suggest that H_2O_2 derived from Nox4 may impinge upon arterial structure.

We recently reported that Polymerase delta interacting protein-2 (Poldip2) binds to p22phox and enhances Nox4 activity.[10] Poldip2 overexpression in VSMC increases ROS production in a Nox4-dependent manner, and knockdown of Poldip2 leads to a loss of focal adhesions and impaired migration. Moreover, overexpression of Poldip2 prevents focal adhesion dissolution in response to PDGF.[11] These observations, together with the known effects of Nox4 on matrix integrity, led us to postulate that Poldip2 may influence vessel contractility and compliance. To test this hypothesis, we generated mice with reduced Poldip2 expression and investigated vascular structure and function. We found that loss of Poldip2 markedly alters aortic extracellular matrix, impairs contractility and increases stiffness, suggesting that the Poldip2/Nox4 axis may be an important regulator of vascular physiology.

Materials and Methods

A detailed Materials and Methods section can be found in the online supplement. Please see http://atvb.ahajournals.org.

Results

Characterization of Poldip2 deficient mice

Mice were produced at the Texas A&M Institute for Genomic Medicine from a clone of embryonic stem cells with a gene trap construct inserted into the first intron of Poldip2 (Supplemental Figure I, A). To verify the location of the insert in vivo, we amplified genomic DNA from a heterozygous mouse using PCR primer pairs P1-P3 and P4-P2

(Supplemental Figure I, A), surrounding both junctions of the construct. Sequencing of the PCR products confirmed the successful interruption of the Poldip2 gene at the first intron and the presence of splice acceptor and β-geo cassette in the correct orientation (not shown).

Aiming to study the physiological effects of a complete Poldip2 ablation in vivo, we crossed heterozygous mice, but obtained very few of the desired homozygous pups (Supplemental Figure I, B). After genotyping 227 weanlings, we only observed 7 live Poldip2−/− mice, rather than 56, as expected from classical Mendelian genetics. Furthermore, these few survivors were small and had to be weaned later than their siblings. This observation suggests that the gene trap effectively blocks expression and that Poldip2 is essential for survival. Since homozygous mice could not be produced in significant numbers, all additional studies were conducted in heterozygotes, which appeared to be healthy and were indistinguishable from their wild-type littermates in size and weight.

To verify the effectiveness of the gene trap and to detect possible changes in Nox subunits, we measured the expression of selected genes by quantitative RT-PCR. While Poldip2 mRNA was decreased by 50% in whole aortas from Poldip2+/− mice, there was no change in p22phox, Nox1, Nox2 and Nox4 (Figure 1A). Reduction in Poldip2 protein expression was confirmed in lysates from whole aortas by Western blotting (Figure 1B), while p22phox and Nox4 proteins were not affected (Supplemental Figure II). Poldip2 protein expression was similarly decreased in lung (not shown). These results suggest that Poldip2 expression is directly dependent on the number of functional genes and further support the idea that the Poldip2 gene trap allele is null. Moreover, because no compensatory upregulation of Nox enzymes was observed, the partial knockdown of Poldip2 can be expected to have detectable physiological consequences.

NADPH oxidase activity in Poldip2+/− mice

Because Poldip2 enhances Nox4 activity,[10] the effect of Poldip2 reduction on NADPH oxidase activity was measured using an electron spin resonance assay. Due to the small amount of aortic tissue available, these measurements were made in kidney slices. As shown in Figure 2, Poldip2 knockdown in heterozygous mice significantly decreased NADPH-dependent production of $O_2^{\bullet -}$ and H_2O_2. As expected, the latter ROS was generated at a much higher rate than the former, because $O_2^{\bullet -}$ produced by Nox4 is mostly converted to H_2O_2, rather than being released.[12, 13] Importantly, these results suggest for the first time that Poldip2 contributes to Nox4 activity in vivo.

Contraction of Poldip2+/− aorta ex vivo

Because vascular contractility is strongly influenced by H_2O_2,[14] the impact of Poldip2 knockdown on vascular contraction was investigated. Isometric force per cross-sectional area was measured in isolated aortic rings exposed to increasing concentrations of the α-adrenergic agonist, phenylephrine. While sensitivity (EC_{50}) to the stimulus was unchanged, maximal force was reduced in vessels from Poldip2+/− mice (48 ± 17 % inhibition) (Figure 3A). This effect was not due to an inhibition of agonist signaling, since it was also observed when contraction was induced by depolarization with exogenous potassium chloride (44 ± 17 % inhibition) (Figure 3B). The inhibition of contraction persisted, albeit to a lesser degree, after removal of the endothelial layer, suggesting that both smooth muscle and endothelium contribute to the observed phenotype (Supplemental Figure III). The inhibition of contraction in Poldip2+/− aortas was also observed when vessels were preincubated for 30 min with 1,000 units of PEG-catalase, indicating that it is not mediated by an acute effect of hydrogen peroxide (Supplemental Figure III). Furthermore, because aortic cross-sectional area was not affected by the genotype (Supplemental Figure IV and data not shown), we explored vascular biomechanics in greater detail. Stiffness appeared to be increased in

maximally dilated aortic rings from Poldip2+/− mice subjected to stepwise extensions (Figure 3C). This result was confirmed by measuring compliance in aortic segments incrementally inflated with cell culture medium (Figure 3D, E).

Structure of Poldip2+/− vessels

To investigate the possibility that the observed changes in compliance were a consequence of structural alterations, we examined transverse aortic sections using transmission electron microscopy. In aortas from wild-type mice, elastic lamellae divide the vascular media in regular concentric rings (Figure 4A and C). In marked contrast, elastic lamellae from Poldip2+/− aorta were frequently fragmented and irregular both in thickness and orientation (Figure 4B and D, Supplemental Figure IV). Furthermore, the percentage of interlamellar area occupied by fibrillar (Figure 4F) or amorphous (Figure 4D) extracellular matrix was significantly increased in Poldip2+/−, compared to wild-type (Figure 4E). These alterations in structural organization are consistent with the decrease in vascular compliance observed in Figure 3 and may relate to abnormal matrix deposition as well as excessive protein secretion by smooth muscle cells.

Collagen I secretion in VSMC

To examine the role of Poldip2 in matrix production, collagen secretion was measured in VSMC isolated from WT and Poldip2+/− mice. As shown in Figure 5, collagen I released into the medium was increased in Poldip2+/− compared to WT cells. A similar result was obtained in rat VSMC treated with Poldip2 siRNA, as extracellular fibronectin and collagen were both increased (Supplemental Figure V). Furthermore, neither the mRNAs of collagens I, III, IV and XVIII, elastin, fibronectin (Supplemental Figure VI) nor intracellular collagen protein were affected (not shown), suggesting that in healthy vessels Poldip2 inhibits secretion, rather than synthesis, of extracellular matrix. To determine whether this effect of Poldip2 on collagen secretion is mediated by ROS, mouse VSMC were treated with exogenous glucose oxidase, which produces H_2O_2 continuously in the presence of glucose in the medium[15] (Supplemental Figure VII). As shown in Figure 5, glucose oxidase abolished the increase in collagen I observed in Poldip2+/− VSMC. Thus, collagen I secretion appears to be inversely related to H_2O_2 production and Poldip2 expression.

To begin investigating other possible changes that may be associated with impaired contraction and elevated extracellular matrix production, the mRNA expression of candidate genes was measured by quantitative RT-PCR. As shown in Supplemental Figure VI, none of the measured inflammatory markers were affected. In contrast, MMP2 and MMP9 were upregulated, while their inhibitors TIMP1 and TIMP2 were unchanged. This result suggests that compensatory mechanisms mitigate the elevation in extracellular matrix deposition in the vessel wall. Further studies will be required to determine which signaling pathways are responsible for this effect.

Blood pressure in Poldip2+/− mice

Impaired vascular contraction and compliance in vitro would be expected to affect blood pressure regulation in vivo. Although basal blood pressure was not changed, Poldip2+/− mice presented a slight reduction in angiotensin II-induced hypertension (Supplemental Figure VIII). This result suggests that Poldip2 can regulate vascular function at least when animals are exposed to a chronic challenge.

Aortic dilatation in Poldip2+/− mice

Because alterations in extracellular matrix deposition would be expected to affect vascular remodeling, aortic dilatation was induced in Poldip2+/− mice by surgical application of

$CaCl_2$ to the abdominal aorta in a model of experimental aneurysm.[16] As expected in vessels with excess matrix, Poldip2+/− mice were protected against vascular dilatation compared to WT mice (Figure 6). Collectively, these findings suggest that a reduction in Poldip2 with a corresponding decrease in Nox4 activity enhances aortic matrix deposition, thereby disrupting vascular structure and leading to reduced agonist-induced contraction and decreased susceptibility to experimental aortic dilatation.

Discussion

This is the first study addressing the functions of Poldip2 in vivo. Although Poldip2 has multiple cellular targets, here we focused on its ability to regulate ROS. We found that Poldip2 ablation is embryonically lethal, whereas a reduction of Poldip2 levels in heterozygous animals decreases NADPH oxidase activity and alters arterial structure. Aortas from Poldip2 heterozygous animals exhibit disordered elastic lamellae and secrete excess collagen, the latter of which appears to result directly from lower H_2O_2 levels. In consequence, smooth muscle contraction and vascular compliance are reduced, which leads to protection against experimental aortic dilatation. Thus, Poldip2 represents a novel mechanism to regulate aortic stiffness, which is a major cardiovascular risk factor.

Poldip2 has been implicated in several signaling pathways essential to life, explaining why Poldip2 knockout is embryonic lethal, while Nox4 knockout is not.[9, 17] Poldip2 is 42-kDa protein with a mitochondrial targeting sequence that is cleaved in some tissues, including smooth muscle, to create a functional 37-kDa protein.[10, 18] It was first identified as a protein that binds to the p50 subunit of DNA polymerase delta and proliferating cell nuclear antigen (PCNA), suggesting that it has a role in DNA repair.[18, 19] Poldip2 was then shown to interact with cell-cell adhesion receptor (CEACAM-1), which enables it to shuttle between the cytoplasmic and nuclear compartments[20] where it has a role in mitotic spindle organization and chromosomal separation.[21] Recently, we found that Poldip2 also interacts with p22phox and increases Nox4 activity, leading to enhanced stress fiber and focal adhesion formation.[10] These latter functions of Poldip2 suggest that it has an important role in cytoskeletal dynamics, which may be related to its ability to interact with Nox4. In this study we found that Poldip2 deletion enhances collagen secretion in a redox-sensitive manner, implying that it may have a concerted effect on matrix-cytoskeletal interactions and vascular contraction.

The clear effect of Poldip2 on vascular structure and extracellular matrix strongly suggested that vascular compliance would be affected. The mechanical properties of isolated aortas were assessed using two different methodologies, which produced similar results. In the first method, force is measured while incrementally stretching aortic rings between two wires. The data indicate that Poldip2+/− arteries are stiffer than wild type, since they require greater force to distend to the same degree (Figure 3C). In the second method, diameter is measured at fixed length, while inflating aortic segments with cell culture medium. In this case, the results also suggest that Poldip2+/− arteries are stiffer than wild type, since greater pressure is required to attain larger diameters (Figure 3D). Although the difference between genotypes appears to be smaller using the second method, it is nevertheless likely to be physiologically relevant because native vessel geometry is better preserved in the latter model.[22–24]

While the decrease in compliance in Poldip2+/− mice seems modest, clinical studies have reported that changes in arterial stiffness can be used as an accurate predictor of risk for cardiovascular events.[25, 26] In other studies, measurements of the incremental modulus of elasticity, distensibility and compliance, showed that markers of stiffness were elevated in patients with end-stage renal disease who died from cardiovascular disease, compared to

those who had no events.[27] It has also been reported that the elastic modulus and Young's modulus were approximately 25% and 11% greater respectively in hypertensive as compared to normotensive patients,[28] and a similar study found that the carotid distensibility in hypertensive patients was approximately 24% lower than in normotensive control patients.[29] Thus, fairly modest changes in arterial stiffness have been associated with cardiovascular disease. The changes in vascular compliance reported in our study (10–14%) are numerically similar to those reported in humans with cardiovascular disease (11–25%). Importantly, it was also shown that the carotid distensibility in hypertensive patients was associated with a high Framingham risk score.[29] Thus, we believe that even though modest, the changes in compliance we observed represent a significant and important role for Poldip2+/− in arterial mechanics corroborated by the decrease in angiotensin II-induced hypertension observed in Poldip2+/− mice (Supplemental Figure VIII).

Although excessive oxidative stress has been linked to fibrosis and increased collagen synthesis in other settings,[30–34] in the present study extracellular matrix and collagen are increased as a result of lower Poldip2 and H_2O_2. Because Poldip2 deletion affects neither the mRNAs of major extracellular matrix components (Supplemental Figure VI), such as collagen I, nor intracellular mature procollagen protein, our findings suggest that reduced H_2O_2 favors procollagen I secretion (Figure 5 and Supplemental Figure V), thereby impairing vascular contraction. This interpretation is supported by the observations that supplementation with H_2O_2 normalized collagen secretion from VSMC over three days, whereas a short preincubation with catalase had minimal effects on vascular contraction. In addition, we observed an increase in matrix metalloproteinases MMP2 and MMP9 mRNAs in Poldip2+/− aortas, with no change in TIMP1 and TIMP2 (Supplemental Figure VI), suggesting feed-back regulation of the excess in extracellular matrix deposition. Future studies will be required to verify that the activity of these proteases is indeed upregulated and to analyze their regulatory pathways in Poldip2+/− tissues.

Poldip2 knockdown also has a dramatic effect on the integrity of the elastic lamellae. Mature elastin fibers are composite structures, consisting of an inner amorphous core of crosslinked elastin and a meshwork of over 30 elastin-associated molecules,[35] including fibrillins, fibulins, microfibril-associated glycoproteins (MAGPs) and proteoglycans.[36] While the amount of elastin in the vessel does not change after birth,[36] elastin fiber assembly is an ordered, hierarchical sequence of events in which microfibrillar and other proteins serve as templates for the deposition of soluble tropoelastin molecules, which then become highly crosslinked and aggregated into insoluble filamentous structures. Progressive aggregation and fibrillogenesis have been linked to the dynamic motility of cells as they move and organize fibrillar material within the mechanically coupled cell-ECM environment.[37, 38] The proposed correlation between cell/tissue motion and the organizational patterning of the ECM scaffold could explain why Poldip2+/− mice, whose VSMCs exhibit impaired focal adhesion and actin cytoskeleton formation and migration,[10] have altered elastin fiber architecture leading to reduced vessel elasticity and potentially to homozygous lethality.

It is not surprising that the contractility and stiffness of vessels from Poldip2 heterozygous mice are compromised, given these major alterations in ECM composition, structure and previously observed reductions in focal adhesions. However, H_2O_2 can also affect contractility of smooth muscle directly or indirectly via release of endothelium-dependent factors.[14] For example, H_2O_2 increases calcium flux through L-type calcium channels, leading to contraction of cerebral arteries,[39] and ROS derived from Nox2 activate protein kinase C to induce contraction in coronary arteries.[40] The combination of increased cytosolic calcium concentration and activation of other signaling kinases can lead to activation of myosin light chain kinase, favoring contraction.[14] Based on these reports, one would predict that the reduction of H_2O_2 in Poldip2+/− animals would lead to less force

generation, which is in fact what we observe. Since our results (Supplemental Figure III) implicate both endothelium and smooth muscle, further studies using tissue-specific Poldip2 knockout animals will be required to dissect the contributions of these potential mechanisms to the reduced force generation observed in Poldip2 heterozygote aortas.

The relative amounts and organization of elastin, collagen, and VSMCs in the media determine the mechanical properties of the tissue. Elastin is generally thought to endow distensibility to tissues over low loading, while collagen provides a stiffener response at higher loads.[41] A mechanistic link between ROS and arterial stiffness has recently emerged. Reduction of vascular H_2O_2, either by deletion of one allele of SOD2[3] or by overexpression of catalase[4] leads to increased collagen deposition and increased aortic stiffness. These results are similar to those observed here in Poldip2+/− aortas, and are consistent with alterations in ROS levels playing a causal role in this animal model. Poldip2+/− animals also exhibit fragmented and disordered elastic lamellae and increased collagen content, which is consistent with increased arterial stiffness. The observed increase in aortic stiffness in these animals suggests that genetically-based reductions in Poldip2 expression or activity in humans may be a risk factor for future cardiovascular disease.

However, loss of Poldip2 can also be protective. Here, we demonstrate that heterozygosity of Poldip2 slightly reduces angiotensin II-induced hypertension and protects animals against aortic dilatation. Both elevations in ROS[42, 43] and dysfunctional elastin and collagen cross-linking[44] promote aneurysm formation. Moreover, alteration in the microarchitecture of adventitial collagen fibrils can contribute to aneurysms.[45] As Poldip2 heterozygote animals have both reduced ROS and increased collagen, either or both pathways could contribute to protection against experimental aortic dilatation.

In summary, we have shown that Poldip2 regulates ROS production in vivo and that deletion of Poldip2 leads to a profound structural alteration in the vessel wall. The induction of collagen secretion by loss of Poldip2 suggests that one perhaps unexpected function of Poldip2 and possibly Nox4 is to repress ECM formation. This previously unidentified function of the enzyme complex has important implications not only for the development of vascular lesions, but also for a number of connective tissue diseases such as rheumatoid arthritis, systemic lupus erythematosus and systemic sclerosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Significance

Polymerase delta interacting protein 2 (Poldip2) was previously shown to enhance Nox4 activity and regulate cytoskeletal organization in vascular smooth muscle cells.[10] We now demonstrate that in vivo knockdown of Poldip2 inhibits NADPH oxidase activity and induces an unexpected vascular phenotype. It has been well documented that oxidative stress, resulting from a pathological increase in ROS production, can induce fibrosis in various tissues. In contrast, our results suggest that a moderate chronic impairment in H_2O_2 production increases collagen secretion by vascular smooth muscle cells and enhances extracellular matrix deposition in the vascular wall, thereby contributing to increased vascular stiffness, reduced contractility and protection against aortic dilatation. Our data thus support the view that subtle changes in redox regulation, regardless of their direction, can have a broad effect on the whole organism.

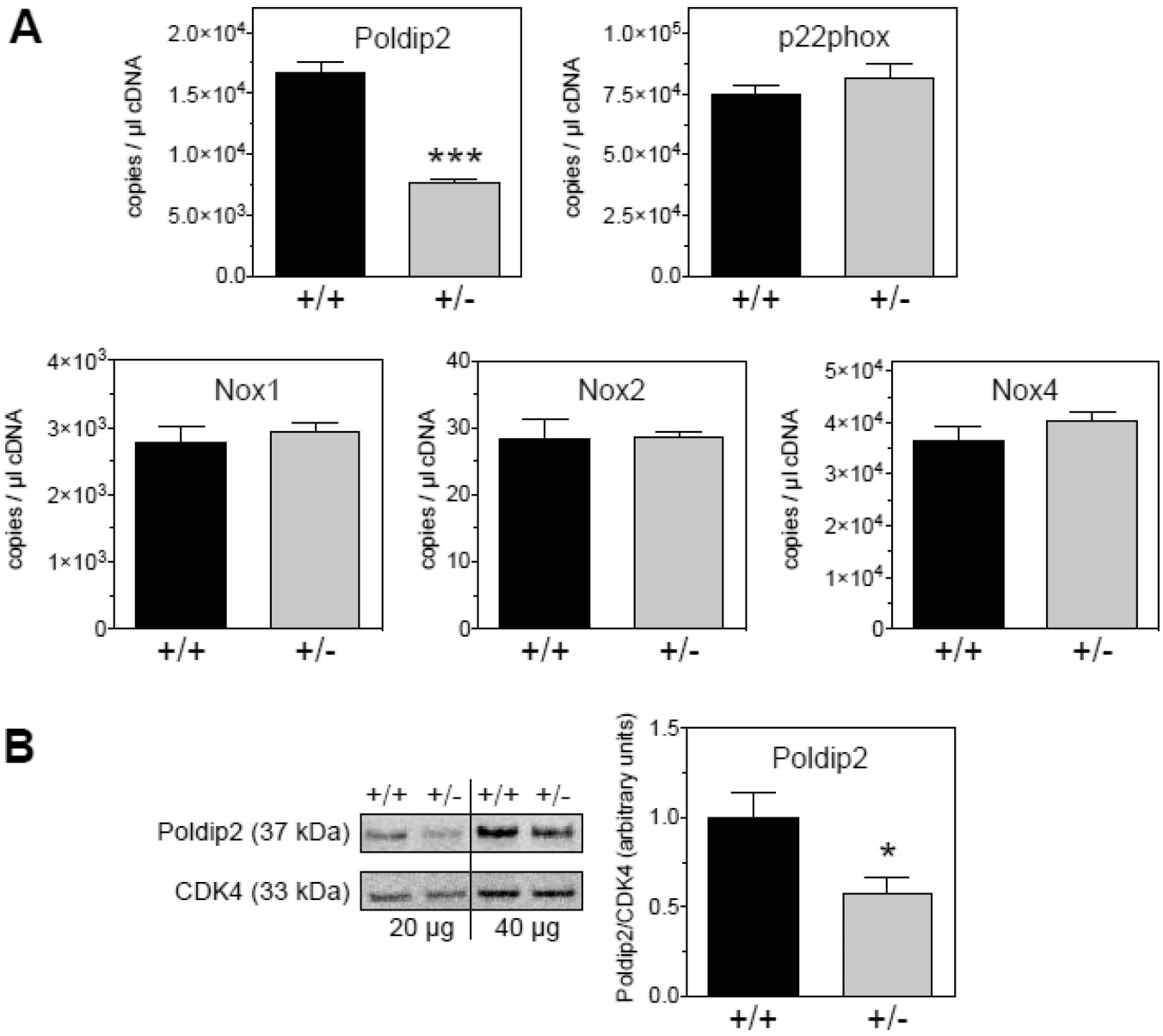

Figure 1. Poldip2 expression is specifically reduced in Poldip2+/− mice

A. mRNAs of Poldip2, p22phox and indicated catalytic subunits of Nox enzymes were measured by quantitative RT-PCR in whole aortas from wild-type (black bars) and Poldip2+/− (gray bars) mice. Data represent average ± SEM from 4 animals; *** $P < 0.001$.

B. Representative Western blots of Poldip2 and CDK4 (loading control) performed using indicated amounts of whole mouse aorta protein (left). Densitometric quantification of Western blot results (right). Data represent averages ± SEM from 7 mice in each group; * $P < 0.05$.

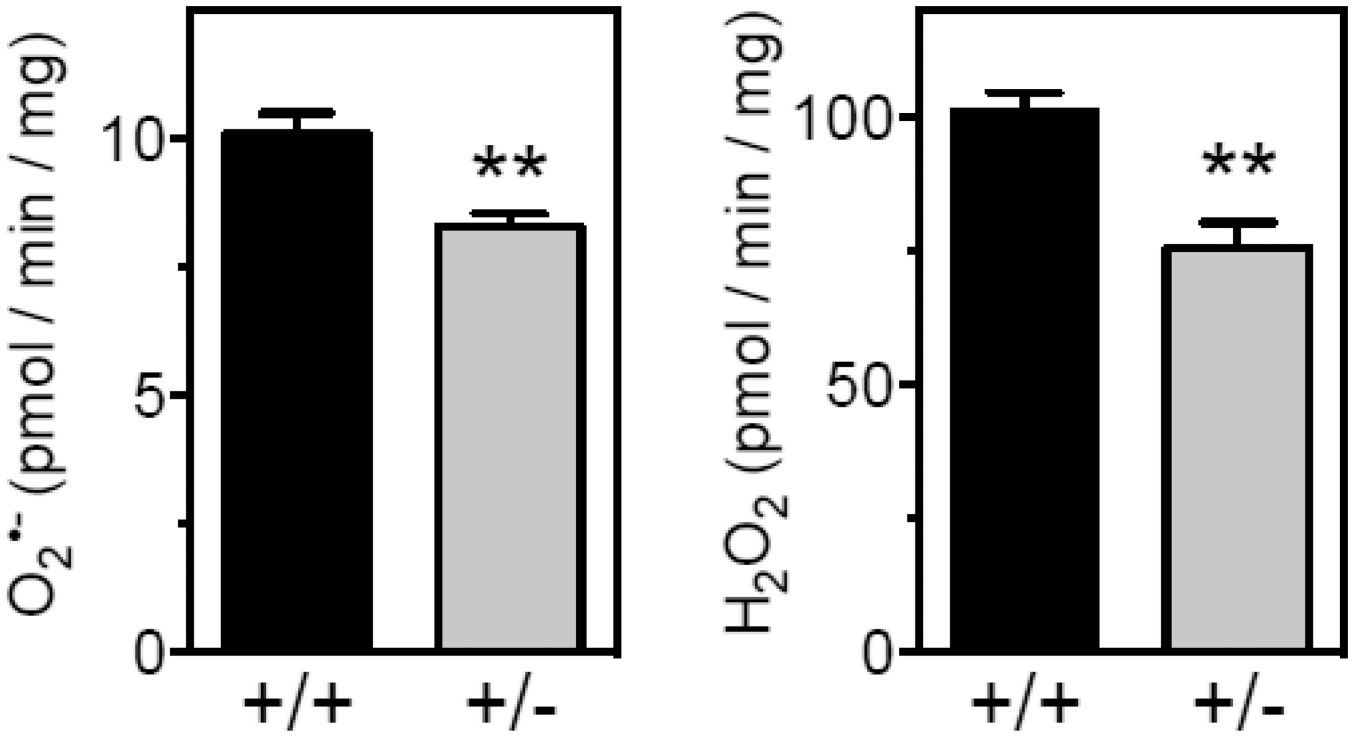

Figure 2. NADPH oxidase activity is inhibited in Poldip2+/− mice

Superoxide ($O_2^{\bullet-}$, left) and hydrogen peroxide (H_2O_2, right) were measured in kidney slices from wild-type (black bars) and Poldip2+/− (gray bars) mice by ESR. Data represent average ± SEM from 5–10 mice in each group; ** $P < 0.01$.

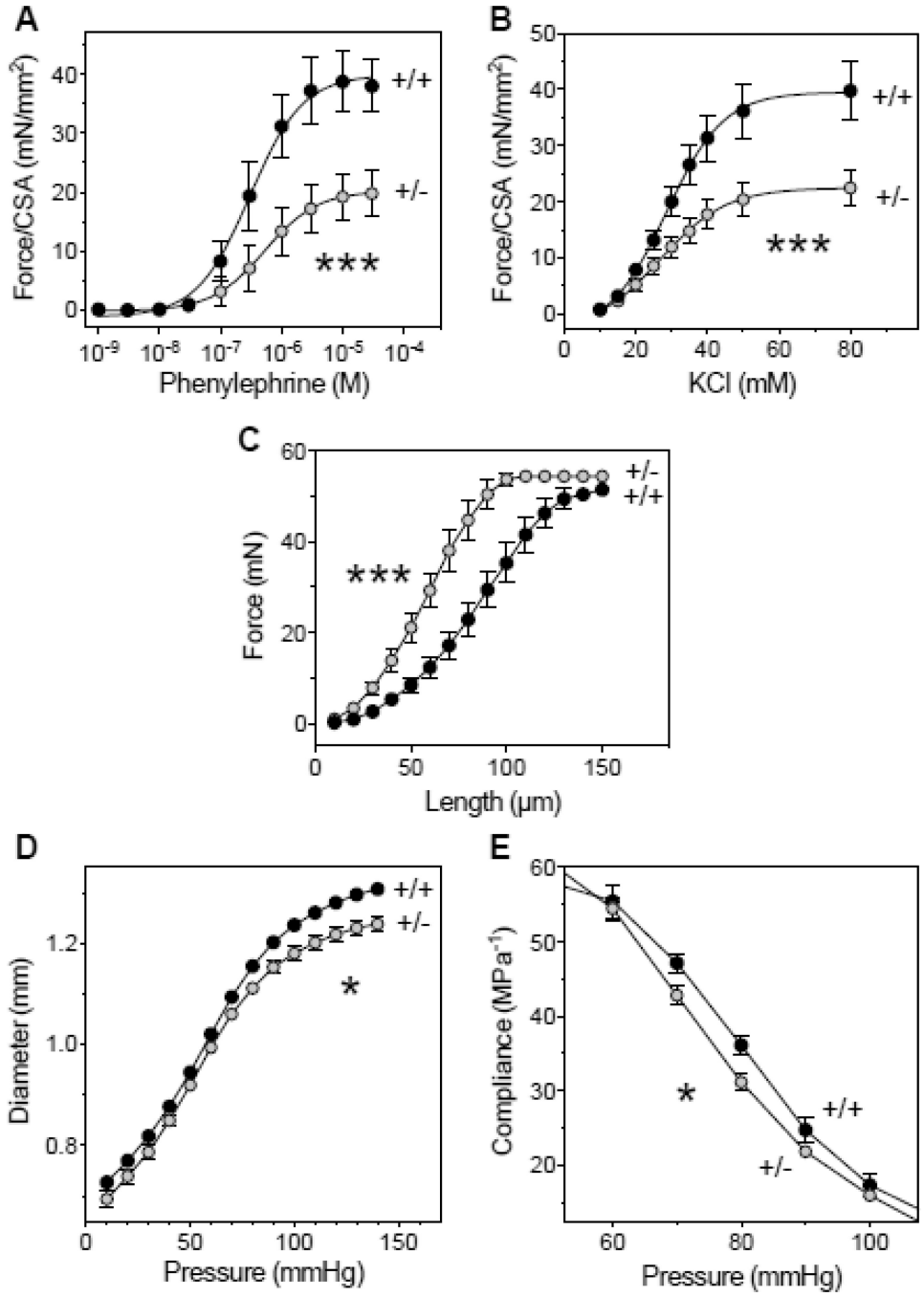

Figure 3. Aortic contraction and compliance are reduced in Poldip2+/− mice

Isometric force normalized to cross-sectional area (CSA) was measured in isolated aortic rings from wild-type (black symbols) and Poldip2+/− mice (gray symbols) exposed to indicated concentrations of phenylephrine (A) or potassium chloride (B). To evaluate stiffness, aortic segments were incrementally elongated (C) or inflated (D, E). Data represent average ± SEM from 5–9 vessels. *** $P < 0.001$, * $P < 0.05$ +/+ vs. +/−.

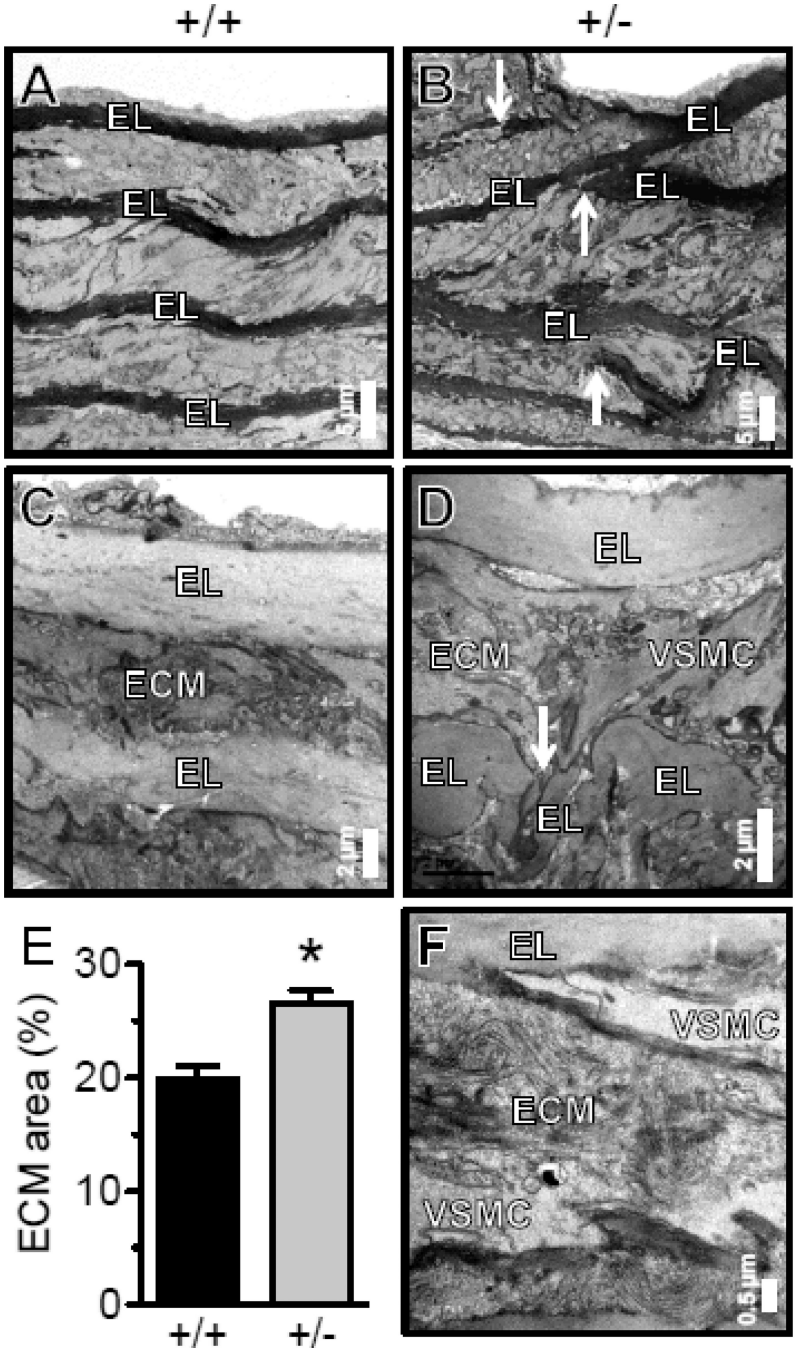

Figure 4. Disrupted elastic lamellae and increased extracellular matrix in Poldip2+/− aorta

Transmission electron micrographs of transverse aortic sections from wild-type (left) and Poldip2+/− (right) mice at increasing magnifications (top to bottom). The vascular lumen is visible at the top of images A–D. Elastic lamellae (EL) appear dark after post-staining with tannic acid in A and B. Breaks in elastic lamellae in Poldip2+/− are marked with arrows in B and D. Morphometric measurements of interlamellar amorphous (C and D) or fibrillar (F) extracellular matrix (ECM) areas in 195 images from 3 wild-type and 3 Poldip2+/− mice are expressed as average ± SEM in E; * $P < 0.05$.

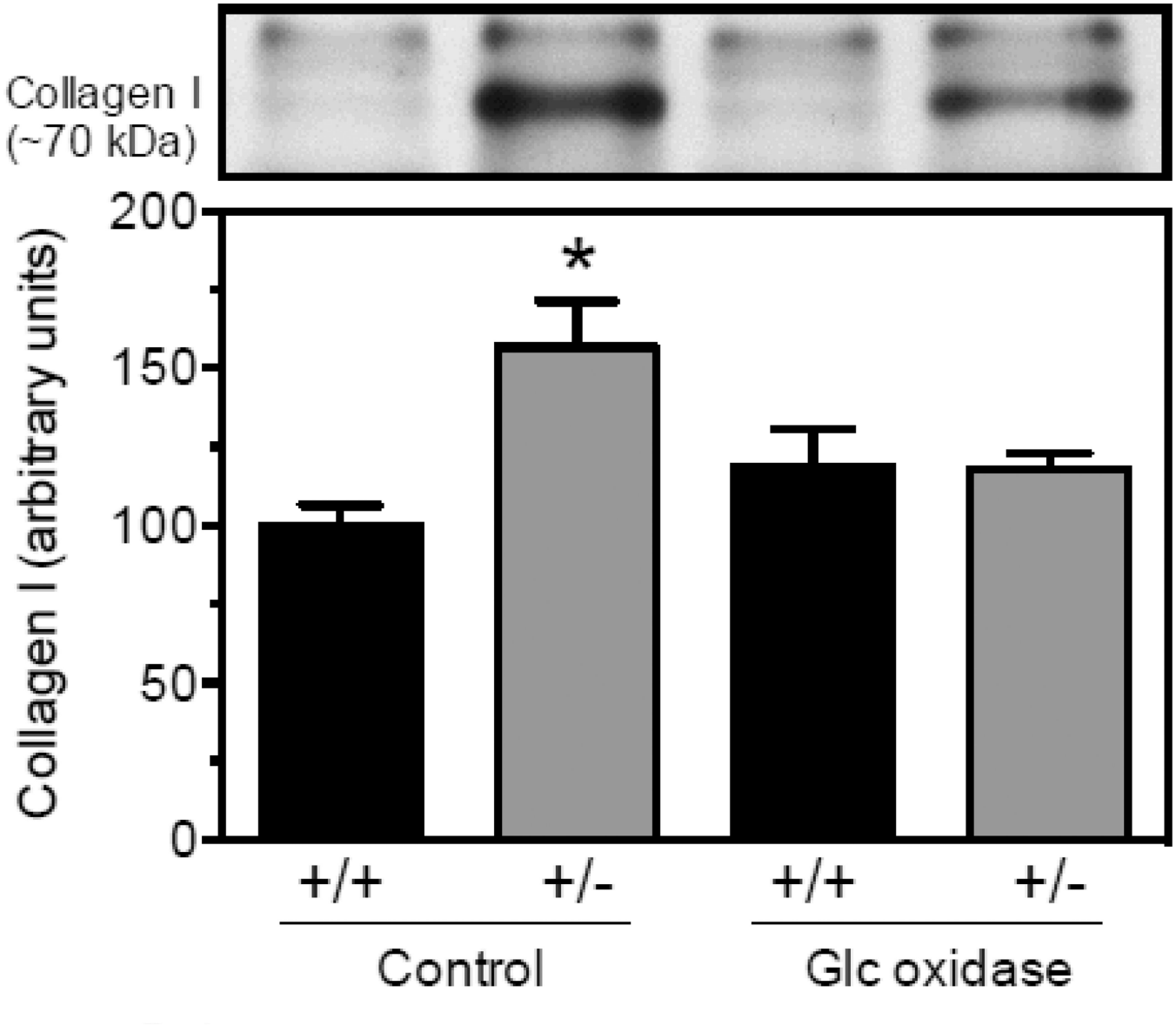

Figure 5. The increase in extracellular collagen I produced by Poldip2+/− VSMC is abrogated by H_2O_2 supplementation

Cultured VSMC from wild-type and Poldip2+/− mice were incubated without (Control) or with 2 ng glucose oxidase for 3 days, before collection of media for Western blot analysis. Equal amounts of protein were loaded in each lane. Representative blot (top) and average densitometric data ± SEM from 9–11 independent experiments (bottom); * $P < 0.05$ vs. +/+.

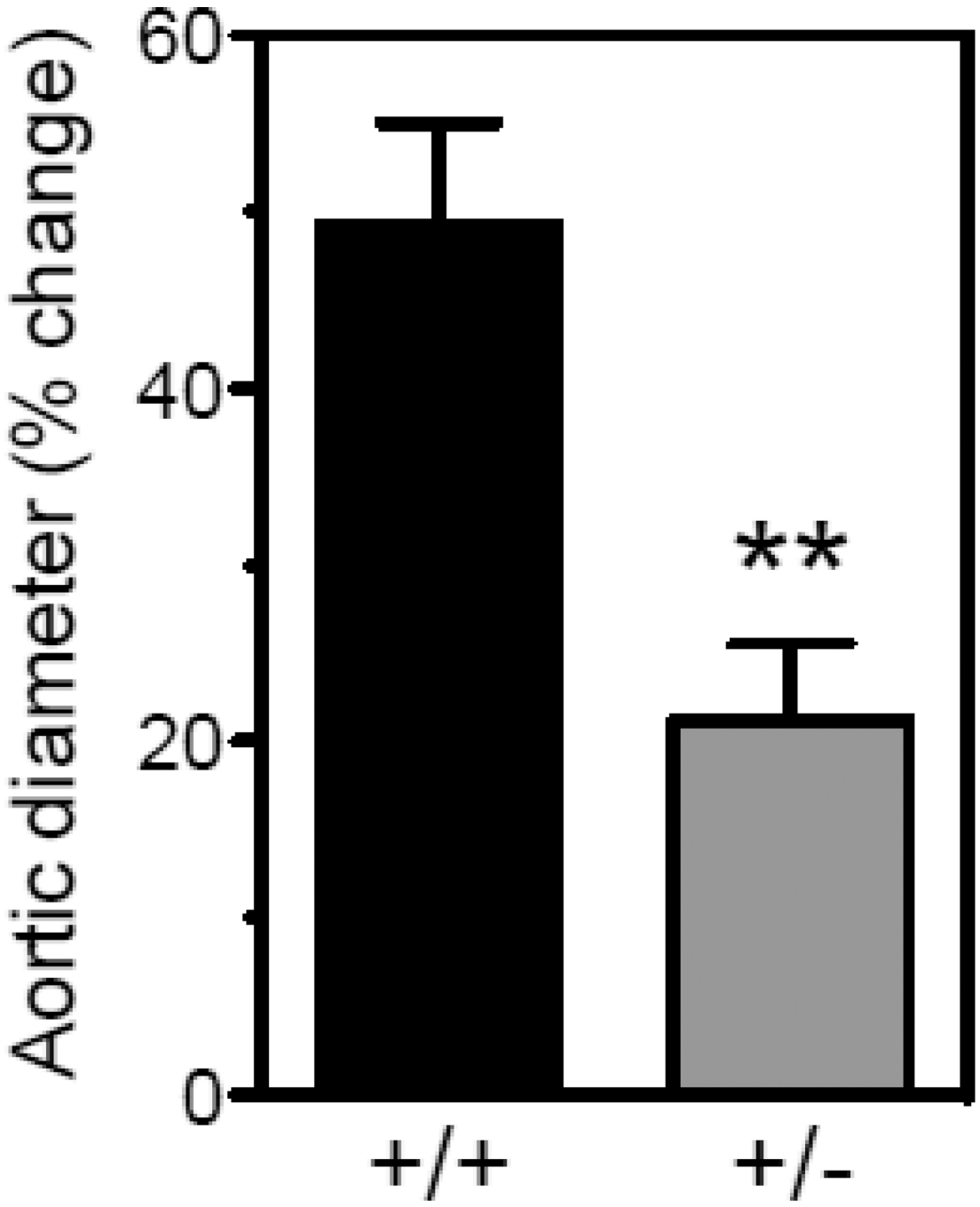

Figure 6. Aortic dilatation is inhibited in Poldip2+/− mice

Dilatation was induced by surgical application of $CaCl_2$ to the abdominal aorta of 9–10 week-old male wild-type (black bar) or Poldip2+/− (gray bar) mice in a model of experimental aneurysm. Aortic diameters were measured before and 8 weeks after treatment and expressed as % change. Data represent average ± SEM from 9–10 mice in each group, ** $P < 0.01$.