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A PROTECTIVE ROLE FOR TIMP-4, A NOVEL PPARγ TARGET GENE, IN SMOOTH MUSCLE IN DOCA-SALT HYPERTENSION

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Abstract

Loss of peroxisome proliferator-activated receptor (PPAR)-γ function causes hypertension, while its activation lowers blood pressure. Evidence suggests these effects may be due to PPARγ activity in the vasculature. However, the specific transcriptional targets of PPARγ in vessels remain largely unidentified. In this study, we examined the role of smooth muscle PPARγ during salt-sensitive hypertension and investigated its transcriptional targets and their functional impact. Transgenic mice expressing dominant negative PPARγ (S-P467L) in smooth muscle cells (SMC) were more prone to deoxycorticosterone acetate (DOCA)-salt-induced hypertension and mesenteric arterial (MA) dysfunction compared to non-transgenic controls (NT). Despite similar morphometry at baseline, vascular remodeling in conduit and small arteries was enhanced in S-P467L following DOCA-salt. Gene expression profiling in aorta and MA revealed significantly decreased expression of *Tissue Inhibitor of Metalloproteinases (TIMP)-4* in S-P467L. Expression of TIMP-4 was increased by DOCA-salt, but this increase was ablated in S-P467L. Interference with PPARγ activity either by treatment with a PPARγ inhibitor, GW9662, or by expressing P467L PPARγ markedly suppressed TIMP-4 in primary SMC. PPARγ binds to a PPAR response element (PPRE) in chromatin close to the TIMP-4 gene in SMC, suggesting that TIMP-4 is a novel target of PPARγ. The interference with PPARγ and decrease in TIMP-4 was accompanied by an increase in total Metalloproteinase (MMP) activity. PPARγ-mediated loss of TIMP-4 increased, whereas over-expression of TIMP-4 decreased SMC migration in a scratch assay. Our findings highlight a protective mechanism induced by PPARγ in DOCA-salt hypertension, establishing a novel mechanistic link between PPARγ and TIMP-4.

Keywords

PPARγ; smooth muscle; vascular remodeling; hypertension; TIMP-4

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Introduction

Thiazolidinediones (TZD) are high affinity synthetic ligands of peroxisome proliferator-activated receptor (PPAR)-γ, a transcription factor of the nuclear hormone receptor superfamily. PPARγ is expressed in adipose tissue, where it regulates gene targets to mediate adipogenesis and fatty acid metabolism. Expression of PPARγ in the vasculature suggests potential cardiovascular actions of PPARγ; however the mechanisms involved remain elusive. In addition to unparalleled effectiveness for improving glycemic control in type 2 diabetes, TZDs have been shown to lower blood pressure and prevent vascular disease.[1,2] Antihypertensive effects of TZD occur in the face of an increase in water and salt reabsorption by the kidney, suggesting robust vascular actions of PPARγ.[3,4] Perhaps, the most intriguing evidence underscoring the significance of PPARγ in blood pressure regulation is from patients with PPARγ mutations. People harboring these mutations (P467L, V290M, R165T and L339X) develop lipodystrophy and severe hypertension due to loss or interference with PPARγ activity.[5,6] Similarly, knock-in mice carrying the mouse equivalent of one of the human mutations (P465L) exhibit hypertension and cerebral vascular dysfunction, hypertrophy, and remodeling, independent of insulin resistance.[7]

PPARγ regulates gene expression by forming a complex with the retinoid X receptor (RXR) and binding to PPAR response elements (PPREs) near target genes. We propose that PPARγ regulates vascular function by altering expression of genes within the vascular wall. Indeed, we reported that a wide array of genes was induced by a PPARγ agonist, while being repressed by dominant negative PPARγ in mouse aorta.[8] To explore the cell-specific actions of PPARγ, we generated a mouse model harboring dominant-negative PPARγ (human PPARγ P467L), the same mutation which causes human hypertension, under control of the smooth muscle myosin heavy chain (SMMHC) promoter (termed S-P467L). Our data indicate that smooth muscle PPARγ is critical in regulating vascular function in conduit and resistance arteries.[9–12] Using gene expression profiling and extensive target validation, we identified RhoBTB1 and Regulator of G-protein signaling 5 (RGS5) as novel PPARγ targets in the blood vessel.[10,11] However, other PPARγ target genes in cardiovascular tissues remain largely unexplored. More importantly, their functional roles during disease conditions need to be examined.

Herein, we tested the hypothesis that smooth muscle PPARγ protects against DOCA-salt-mediated hypertension and resistance vessel dysfunction. We used mild DOCA-salt hypertension to unmask the genetic predisposition caused by the loss of smooth muscle PPARγ. Our findings uncovered Tissue Inhibitor of Metalloproteinases (TIMP)-4 as a novel PPARγ target and highlight the protective mechanism of PPARγ during hypertension.

Methods

Pressurized myograph, quantitative real-time RT-PCR (qRT-PCR), primary cell culture studies, Western blotting, in situ zymography, chromatin immunoprecipitation (ChIP) and scratch assay were performed as described in the online only supplement.

Animals

Transgenic mice (4–5 months old) carrying a dominant negative PPARγ P467L mutation under the control of *SMMHC* (S-P467L) were used.[9] Male mice were used since the blood pressure (BP) rise in female mice is slower and less pronounced with DOCA-salt.[13] Mice were subcutaneously implanted with DOCA pellet (50 mg) and allowed ad lib. 0.15 M NaCl for 21 days in addition to regular chow and water. Arterial BP was measured using radiotelemetry.[9] Care of the mice met the standards set forth by the National Institutes of Health (NIH) guidelines for the care and use of experimental animals. All procedures were approved by The University of Iowa Animal Care and Use Committee.

Data analysis and statistics

Data were expressed as mean±SEM and analyzed with SigmaStat (Systat Software). Repeated-measures analysis of variance (2-way ANOVA) using Bonferroni post-hoc tests for multiple-comparisons was used for vascular reactivity data. Student's *t*-test was used as required. Gene expression changes were calculated by the Livak method.[14] $P \leq 0.05$ was considered significant.

Results

A significant increase in heart and kidney weights was observed in NT and S-P467L mice after DOCA-salt. There was no difference in body, heart or kidney weights between genotypes either before or after DOCA-salt (Table S1). Renal renin mRNA was robustly suppressed after DOCA-salt in both groups (Figure S1).

Arterial pressure was similar in both NT and S-P467L mice at baseline (Figure 1A). This differed from previous cohorts of S-P467L mice, which exhibited a modest increase in baseline arterial pressure.[9,10,15] After DOCA-salt, there was an increase in mean arterial pressure (MAP) in both NT and S-P467L mice, but the DOCA-salt-induced increase in MAP in S-P467L was significantly greater than in NT (Figure 1A). The peak in MAP at midnight was significantly higher in S-P467L following DOCA-salt (MAP [mmHg]; NT=140.5±2.6, n=13; S-P467L=156.9±4.6, n=11, p=0.004). As reported previously, S-P467L mice exhibited tachycardia due to impaired baroreflex regulation.[15] DOCA-salt treatment resulted in a similar degree of bradycardia in both groups (Figure 1B).

Vasodilation to acetylcholine (Ach) was slightly impaired in S-P467L mesenteric arteries (MA) at baseline and this effect was further exaggerated after DOCA-salt (Figure S2A). The maximum response to Ach in S-P467L was significantly decreased after DOCA-salt (E_{max} NT=82±3.8%; S-P467L=50±12.5%, $p<0.05$). Vasodilation to nitroprusside was normal in all groups (Figure S2B). Middle cerebral arteries from S-P467L mice exhibited a similar impairment in Ach-mediated vasodilation (Figure S2C).

Neither ET-1 transcript nor ET-1-induced vasoconstriction differed between NT and S-P467L (Figure S3A). In both genotypes, DOCA-salt led to a robust increase in plasma AVP, measured by the C-terminal portion of AVP precursor, Copeptin (Figure S3B).[16] AVP-induced vasoconstriction in S-P467L was modestly reduced with DOCA-salt (Figure S3C). Thus, the exaggerated sensitivity to DOCA-salt-induced hypertension in S-P467L is unlikely

to be due to ET-1 or AVP. Vasoconstriction to KCl and phenylephrine was similar between genotypes (Figure S3D–E).

A previous study reported an upregulation of local renin angiotensin system components from hypertensive patients carrying the R165T and L339X mutations in PPARγ.[6] However, there was no change in aortic expression of angiotensinogen, renin, AT_{1A} receptor, nor AT_{1B} receptor in S-P467L at baseline or following DOCA-salt (Figure S4).

There was no difference in aortic morphometry between genotypes at baseline (Figure S5). However, wall thickness in aorta from S-P467L mice was markedly increased after DOCA-salt (Figure 2A). Similarly, there was a significant increase in wall thickness and the media/lumen ratio (Figure 2B), and a trend toward an increase in media cross sectional area of small blood vessels in DOCA-salt treated S-P467L, despite similar morphometry of small arteries under baseline conditions.[11] These data suggest that loss of smooth muscle PPARγ function facilitates vascular remodeling in hypertension.

We investigated the expression of MMPs and their endogenous inhibitors, TIMPs, as they are reported to participate in vascular remodeling in hypertension [17,18]. A significant increase in MMP-9, but not MMP2, mRNA was observed in S-P467L arteries after DOCA-salt (Figure 3A). Notably, there was a significant decrease in TIMP-4 mRNA in arteries from DOCA-salt-treated S-P467L, whereas the expression of other TIMPs was unchanged (Figure 3B). This is consistent with a reanalysis of our published microarray datasets (NCBI Accession GSE37195 & GSE36482) showing a significant decrease in TIMP-4 mRNA in aorta and MA from S-P467L mice (Figure 3C). There was a significant down-regulation in TIMP-4 expression in small arteries from S-P467L at baseline (Figure 3D). TIMP-4 mRNA expression was increased in NT arteries following DOCA-salt, but this increase was severely blunted in S-P467L mice (Figure 3D).

GW9662, a PPARγ inhibitor decreased TIMP-4 protein expression in primary vascular SMCs suggesting the decrease in TIMP-4 expression in S-P467L mice was attributable to interference with PPARγ (Figure 4A). This effect was only consistently observed with 10 μM, possibly due to the short half-life of GW9662 in cell culture.[19] To exclude PPARγ independent mechanisms, we demonstrated that expression of TIMP-4 was markedly suppressed in SMC transiently transduced with adenovirus encoding P467L PPARγ (Ad-P467L, Figure 4B). We also generated primary SMC cultures from MA of mice carrying a transgene consisting of 1) the CAG promoter/enhancer followed by a STOP signal (loxP-STOP-loxP), 2) P467L PPARγ, and 3) the tdTomato gene downstream of an IRES element. Ad-Cre treatment of these cells led to Cre-mediated removal of the STOP signal followed by induction of tdTomato fluorescence (data not shown), human PPARγ mRNA (average CT=28.35, n=3), and PPARγ protein (Figure 4C). We previously showed that expression of RGS5 and Prkg2 was robustly altered in MA from S-P467L mice, and this was recapitulated in Ad-Cre infected cells (Figure S6).[11] Expression of TIMP-4 was markedly downregulated in cells after induction of P467L PPARγ (Figure 4C).

To determine if TIMP-4 is direct transcriptional target of PPARγ we searched for PPARγ binding sites (PPREs) within 10 kb of the TIMP-4 transcriptional start site. Two potential

PPREs were identified downstream of TIMP-4, within intron 4 of the SYN2 gene in which the entire TIMP-4 gene is embedded (Figure 5A).[20,21] Expression of SYN2 was not altered in S-P467L (fold change from NT; aorta: 0.96, p=0.5; MA: 0.99, p =0.6), suggesting that regulation of TIMP-4 expression is distinct from SYN2. Both PPREs are conserved and show high similarity with a consensus PPRE (Figure 5A). The binding of endogenous PPARγ (Ad-GFP) or in Ad-P467L-transduced PPARγ at the PPRE closer to TIMP-4 promoter (PPARG-1) was examined in SMC by ChIP. The P467L mutation does not impair DNA binding and has been reported to stabilize the co-repressor complex.[22] As a control, P467L PPARγ physically interacted with a PPRE at the FABP-4 locus, a known PPARγ transcriptional target (Figure 5B). Binding of PPARγ at the PPARG-1 PPRE downstream of TIMP-4 was observed (Figure 5C), consistent with ChIP-Seq studies in 3T3-L1 adipocytes (NCBI Accession GSE13511).

Given that TIMP-4 is an inhibitor of MMP, we evaluated total MMP activity following PPARγ inhibition using *in situ* zymography. The same concentration of GW9662 that caused a robust reduction of TIMP-4 significantly increased net MMP activity in SMC (Figure 6A). Inhibition of MMP activity using EDTA significantly blunted the fluorescent signal, suggesting that the enzymatic activity observed is specific to MMP. We next used the scratch assay to examine cell migration because it allows direct evaluation of cell-matrix and cell-cell interactions.[23] SMC-transfected with human TIMP-4 migrated significantly slower than those with empty vector (Figure 6B). TIMP-4 did not affect SMC proliferation as assessed by expression of a cell proliferation marker (proliferating cell nuclear antigen, Figure S7A). Stably expressing mutant P467L PPARγ by Cre-mediated activation of inducible PPARγ (and tdTomato) transgene in mesenteric SMC downregulated TIMP-4 (Figure 6C) and led to a robust increase in migration compared to SMC derived from a similar Cre-inducible transgene overexpressing wildtype (WT) PPARγ (Figure 6C), without affecting cell proliferation (Figure S7B).

Discussion

Originally known for its requirement in adipogenesis, PPARγ also exerts specific functions outside adipocytes. Targeted inhibition of PPARγ activity in smooth muscle or endothelial cells leads to vascular dysfunction and changes in BP thus underscoring the direct actions of PPARγ in the vasculature.[2] However, it remains unclear what genes are regulated by PPARγ and the mechanisms by which they confer cardiovascular protection. The current study highlights the significance of smooth muscle PPARγ during salt-sensitive hypertension. The main conclusion of the study is that interference with smooth muscle PPARγ predisposes to vascular dysfunction and remodeling during DOCA-salt induced hypertension through a mechanism involving robust down-regulation of a novel PPARγ target, TIMP-4 and increased MMP activity. Our data is consistent with previous studies showing that TIMP-4 protein expression was significantly increased in the medial layer of the rat carotid artery undergoing vascular injury, and that overexpression of TIMP-4 resulted in inhibition of SMC migration without affecting proliferation.[24,25]

We previously demonstrated that smooth muscle PPARγ is important in maintaining arterial function, baroreflex and systemic BP using S-P467L mice.[9–12,15] However, unlike previous

cohorts, we did not observe an increase in BP in S-P467L mice at baseline in the current study even though we examined multiple independent cohorts of S-P467L mice. Although reason for this remains unclear, we offer two possibilities. First, the previously reported increase in arterial pressure was modest (approximately 7–10 mmHg).[9,15] Second, the present cohorts of mice have been backcrossed to C57BL/6 for nearly 20 generations, many more than the mice used in our previous studies. Systemic BP has been reported to be lower in C57BL/6 and the genetic background has been shown to influence BP in other mouse models.[26,27] Regulation of BP is complex and involves a combination of mechanisms including central nervous system, kidney, immune, and vasculature. Interestingly, the tachycardia we previously reported in S-P467L mice was preserved in each of the cohorts used in this study. Similarly, aortic dysfunction and enhanced myogenic tone in mesenteric and middle cerebral artery remain robust in recent cohorts of S-P467L mice (data not shown). Thus, it remains possible that the elevation of BP in S-P467L observed previously is attenuated by some compensatory mechanism. Nonetheless, S-P467L mice are more susceptible to the development of DOCA-salt hypertension.

DOCA-salt hypertension is a low renin model of essential hypertension. Low renin hypertension accounts for about 27% of hypertensive patients and is more prevalent in African Americans.[28,29] Typically, high dose DOCA is combined with reduced kidney mass and high salt intake to induce robust hypertension and consequent end organ damage.[30,31] Herein, we used a “mild DOCA-salt hypertension” protocol which employs low dose DOCA without uninephrectomy which induces a modest and gradual increase in blood pressure.[29–32] The rationale was to unmask a predisposition to hypertension caused by the loss of smooth muscle PPARγ. The role of decreased TIMP-4 in mediating the BP response to DOCA-salt would require further investigation. Interestingly, there is no difference in the pressor response angiotensin II infusion in TIMP-4 knockout.[33]

Our data strongly suggest that the marked reduction of TIMP-4 in S-P467L arteries is a direct consequence of interference of PPARγ activity in smooth muscle, and therefore TIMP-4 is a direct transcriptional target of PPARγ. The evidence for this is two-fold. First, direct inhibition of PPARγ activity in SMC culture either by expressing dominant negative PPARγ or treatment with GW9662 led to a marked down-regulation of TIMP-4. Second, we detected strong binding of PPARγ in chromatin at a PPRE in close proximity to the TIMP-4 gene in SMC. Based on this result, it was surprising that TZD treatment of primary SMC does not result in induction of TIMP-4 expression (data not shown). This suggests that PPARγ may simply be required for the maintenance of basal TIMP-4 expression. This conclusion is supported by the observation that dominant negative PPARγ prevents the induction of TIMP-4 by DOCA-salt. It is important to note that induction of PPARγ by TZDs both induces and represses gene expression. Such an effect has been reported in both adipocytes and in aorta by us.[8,34] Indeed, not all PPARγ target genes are uniformly induced by TZD. Indeed, the adverse effects of TZDs are very likely due to massive activation of certain genes in kidney, bone and heart. In some cases, like TIMP-4, PPARγ may be required to maintain a basal level of expression. However, like those gene induced by TZDs, expression of genes like TIMP-4 will be impaired by mutants of PPARγ which cause human disease. This is because these mutations increase the occupancy of PPARγ on the PPRE and do not allow dismissal of co-repressors.[35] It is also possible that other transcription factors

in addition to PPARγ regulate TIMP-4 expression. Consistent with this, preliminary data from our laboratory suggested that TIMP-4 expression could be induced by aldosterone treatment in vascular SMC cultures. Genome-wide ChIP data from the Encode project provided evidence of binding of glucocorticoid receptor to the human TIMP-4 promoter, and within this same region, we identified two sequences similar to the hormone response element (HRE), which can be bound by either mineralocorticoid or glucocorticoid receptor.

Although the mechanism of PPARγ action has been extensively studied in adipocytes, the transcriptional targets in other tissues remain less well known. We previously unraveled the role of RGS5 in resistance vasculature and identified it as a novel PPARγ target.[11] Recent studies have demonstrated beneficial actions of TZDs on vascular function in preeclampsia through upregulation of RGS5, highlighting the importance of understanding the transcriptional targets of PPARγ in different tissues.[36] We cannot exclude the possibility that other genes beside TIMP-4 altered by interference with PPARγ activity in SMC also contribute to exaggerated DOCA-salt-induced hypertension and vascular remodeling. Moreover, changes in other genes such as an induction of MMP-9 in S-P467L blood vessel after DOCA-salt also likely contributes to increased vascular remodeling. Although the inhibitory effect of TIMP-4 on SMC migration appears modest, its significance is likely amplified during disease conditions. In particular, the failure to increase TIMP-4, attributable to the loss of smooth muscle PPARγ, and the concurrent increase in MMP-9 after DOCA-salt can cause an imbalance in total MMP/TIMP activity, favoring increased SMC migration and vascular remodeling. In line with this, TIMP-4-deficient mice exhibit high mortality rates following myocardial infraction. [37]

Perspectives

Arterial remodeling occurring during hypertension and type-2 diabetes contributes to end-organ damage and serious cardiovascular complications.[38,39] Activation of PPARγ by TZD reduces vascular hypertrophy, lowers systemic BP and lessens cardiovascular risks.[40] Conversely, loss of PPARγ function results in enhanced smooth muscle migration and small blood vessel remodeling by unclear mechanisms.[7,41] Accumulating evidence indicates that PPARγ exerts vascular protection through regulation of its transcriptional targets in endothelium and SMC. Herein, we demonstrated a novel mechanism by which smooth muscle PPARγ regulates migratory phenotype of smooth muscle cells and arterial remodeling during salt-sensitive hypertension though TIMP-4. We focused on the function of TIMP-4 because its change in expression was among the most robust, and there was a logical rationale for its involvement in vascular remodeling. We validated TIMP-4 as a new PPARγ target and showed that specific suppression of TIMP-4 by mutant PPARγ is functionally associated with increased SMC migration and enhanced total MMP activity (see model in Figure 6D). The failure to increase TIMP-4 caused by defective PPARγ during hypertension was associated with augmented arterial remodeling and an exaggerated increase in BP. Further studies should address whether the beneficial effect of PPARγ activation on BP requires TIMP-4. Impaired PPARγ activity reported frequently in diabetes and hypertension might be associated with reduced vascular TIMP-4 and that might be an underlying mechanism of increased blood vessel stiffness and microvascular remodeling associated with end organ damage. Our current studies shed light on a therapeutic potential

of TIMP-4 during hypertension and provide a previously unrecognized link between PPARγ and TIMP-4.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Novelty and Significance

What Is New?

- Transgenic mice expressing a mutation in PPARγ selectively in SMC exhibited increased sensitivity to DOCA-salt-induced hypertension and vascular remodeling.
- Augmented vascular remodeling in these mice was associated with suppressed TIMP-4 expression.
- The expression of TIMP-4 was inversely correlated with the level of SMC migration.
- We identified TIMP-4 as a new PPARγ target gene in SMC.

What Is Relevant?

- Activation of PPARγ by TZD reduces blood pressure, but loss of PPARγ function is associated with hypertension. However, the mechanism remains unclear.

Summary

- Our current studies identify a potential mechanism by which PPARγ exerts vascular protective effects in hypertension and vascular injury through activation of TIMP-4. Impaired PPARγ causes a decrease in TIMP-4, and increase in MMP-9 leading to remodeling.

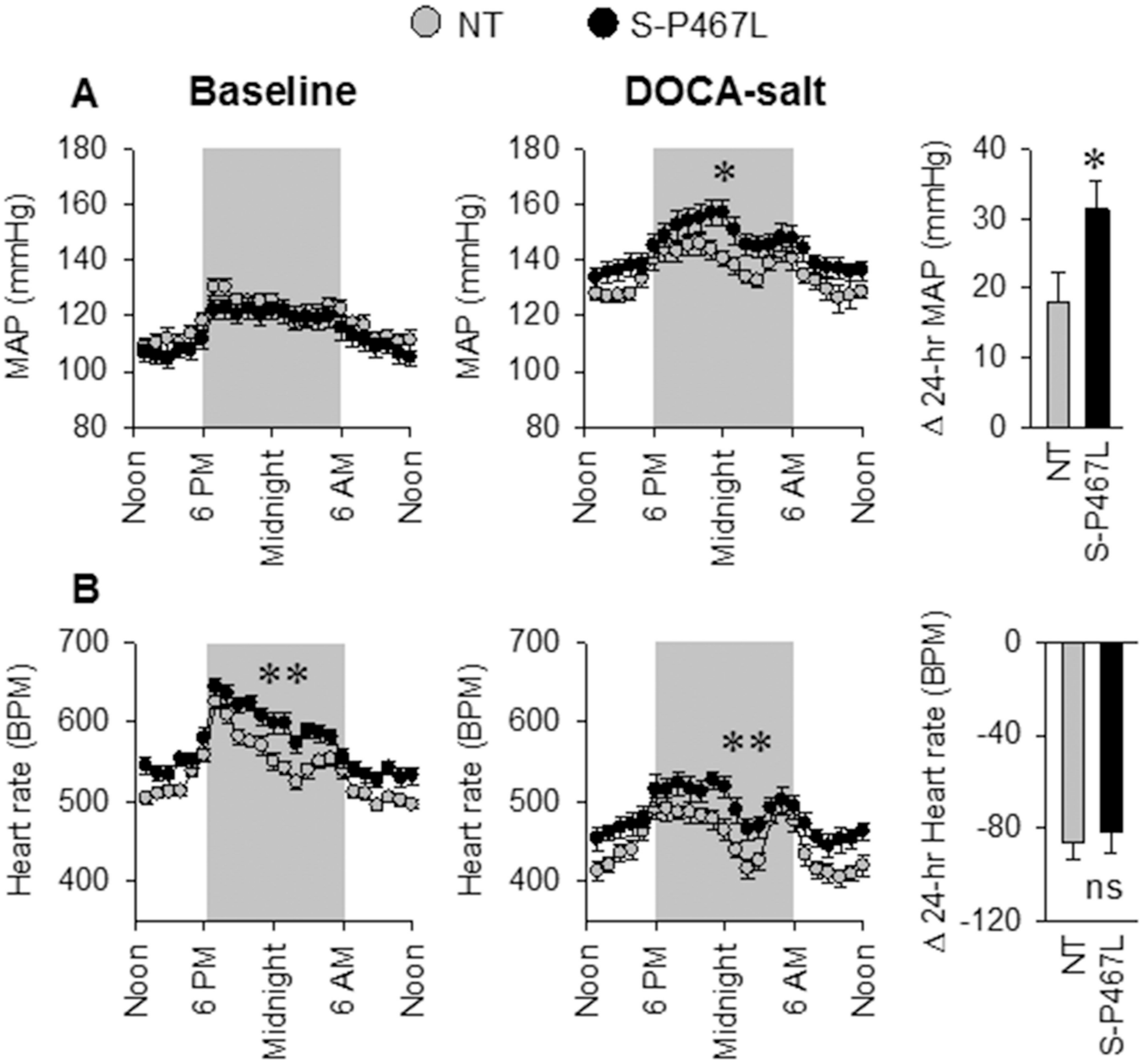

Figure 1. Arterial pressure response to DOCA-salt in S-P467L mice

A) Mean arterial pressure (MAP) and B) heart rate at the baseline and after DOCA-salt treatment. Each data point is an average of each hour from seven days recording. (NT baseline, n=16; S-P467L baseline, n=13; NT+DOCA-salt, n= 13; S-P467L+DOCA-salt, n=11). 2-way RM-ANOVA Genotype X Treatment interaction P= 0.039 for MAP. *p<0.05 compared to NT MAP; **p<0.05 compared to NT heart rate. NS=non-significant.

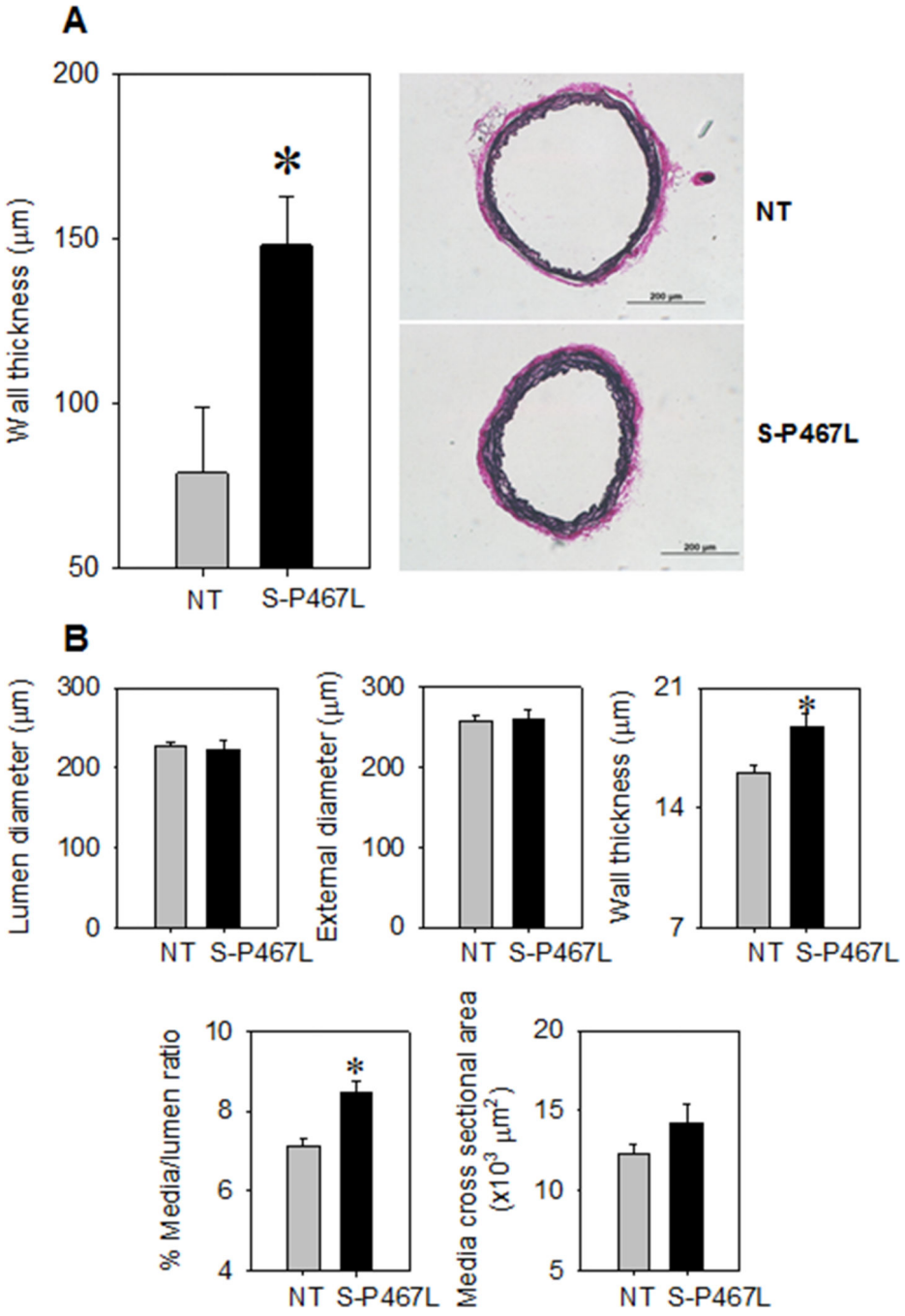

Figure 2. Augmented vascular remodeling in S-P467L after DOCA-salt

Structural parameters of aorta stained with Verhoeff-van Gieson (A, NT n=6; S-P467L n=6) and small mesenteric arteries (B, NT n=12; S-P467L n=8) in Ca^{2+}-free condition (at 75 mmHg) after DOCA-salt. *p<0.05 compared to NT.

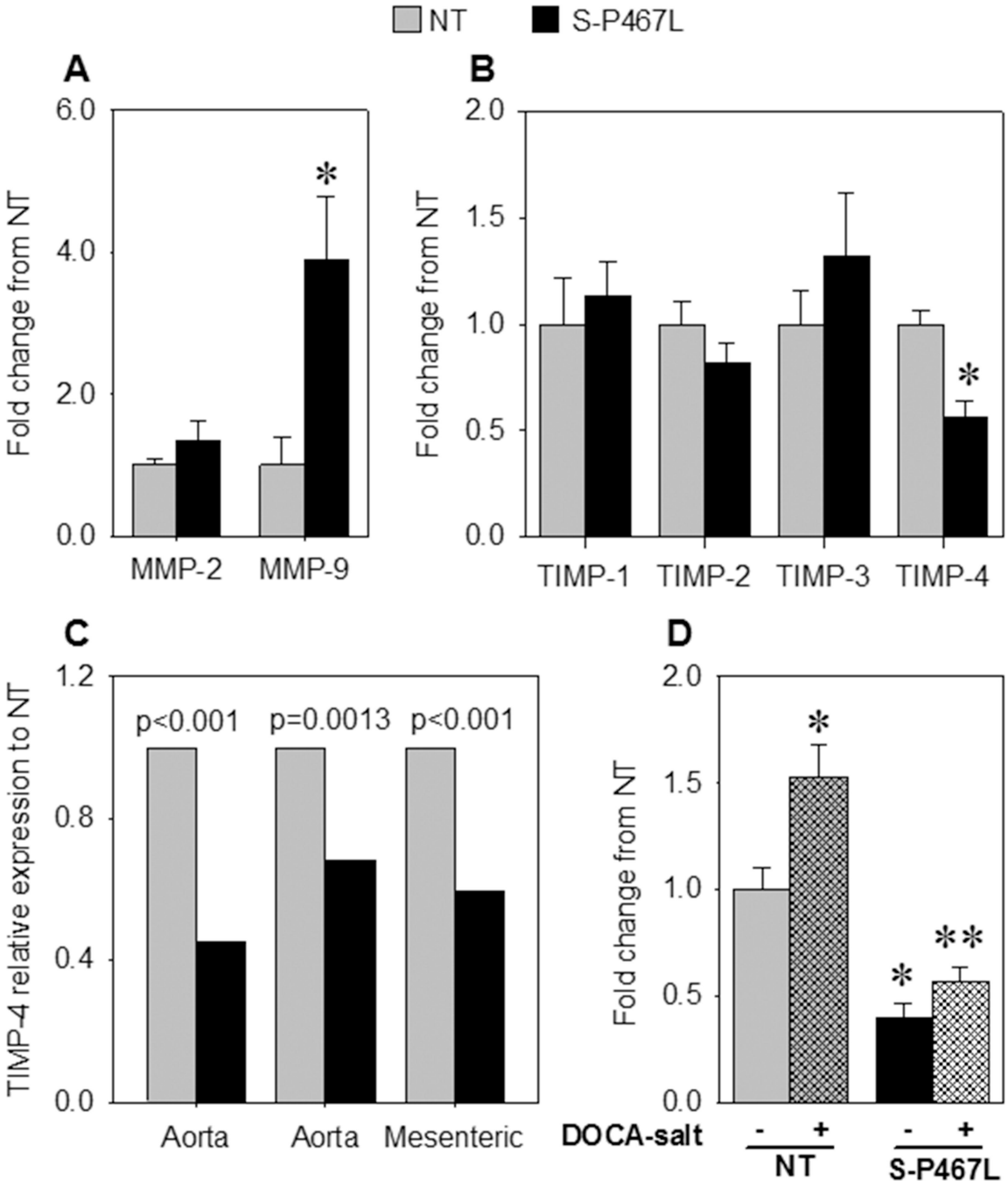

Figure 3. Expression of TIMP-4 and MMP-9 in S-P467L following DOCA-salt

mRNA expression of MMP-2 and −9 (A) and TIMPs (B) in small arteries after DOCA-salt (NT n=5; S-P467L n=6). $p<0.05$ compared to NT. C) Microarray data showing TIMP-4 mRNA expression in aorta and MA. The first bar is data from a oligonucleotide array, whereas the other bars are from an exon tiling array. D) Expression of TIMP-4 in MA before and after DOCA-salt measured by Q-RT-PCR (NT baseline n=6; S-P467L baseline n=6; NT +DOCA-salt n=5; S-P467L+DOCA-salt n=6). *$p<0.05$ compared to NT at baseline; **$p<0.05$ compared to S-P467L+DOCA-salt.

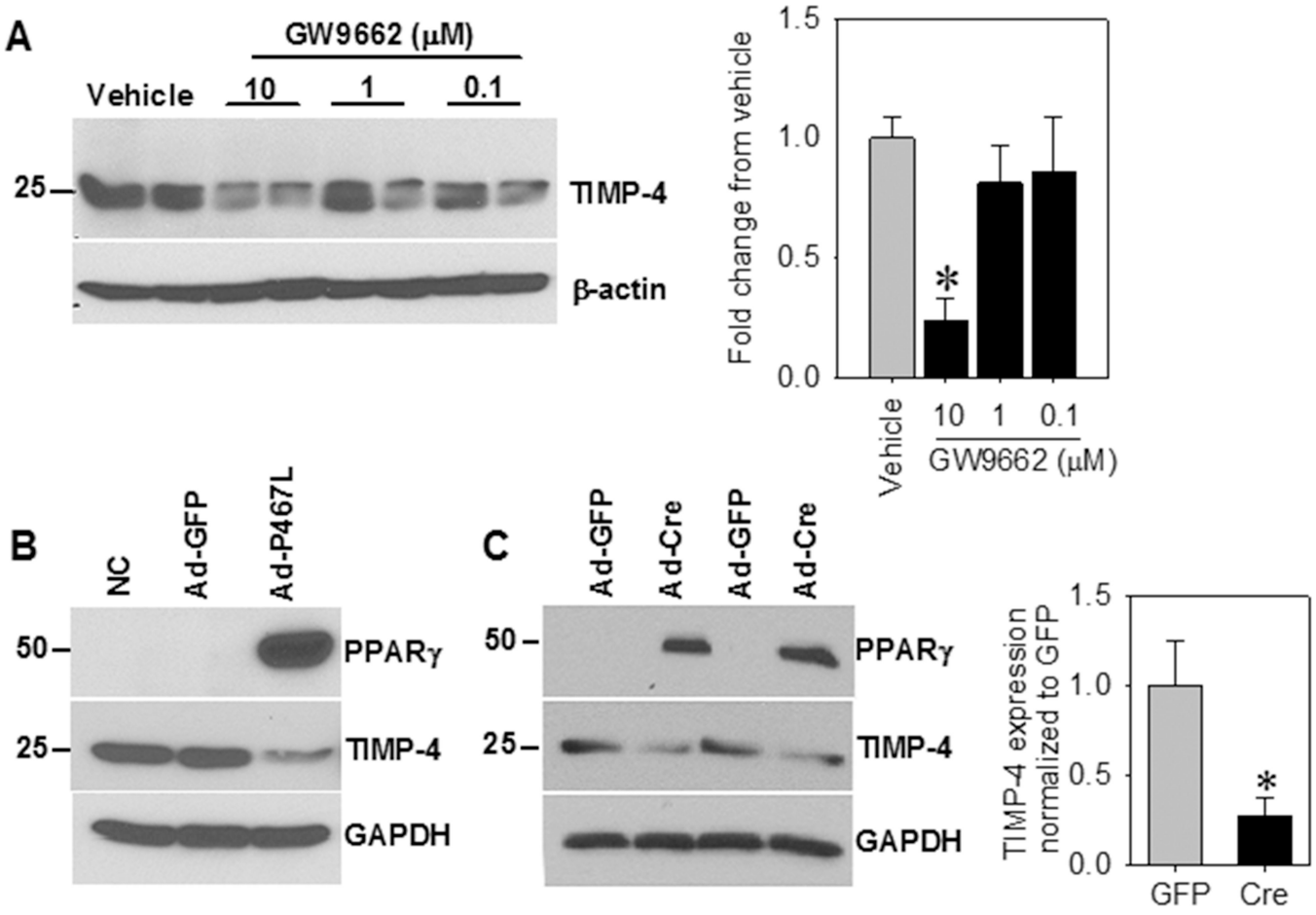

Figure 4. PPARγ interference downregulates TIMP-4 in SMC

A) TIMP-4 protein in primary rat aortic SMC (RASMC) following GW9662 (n=4). B) PPARγ and TIMP-4 protein 48 hours after transduction of Ad-GFP or Ad-P467L in RASMC (n=3). C) PPARγ and TIMP-4 protein from primary MA SMC derived from transgenic mice conditionally expressing P467L PPARγ in response to Cre-recombinase (n=5). *p<0.05 compared to vehicle or Ad-GFP.

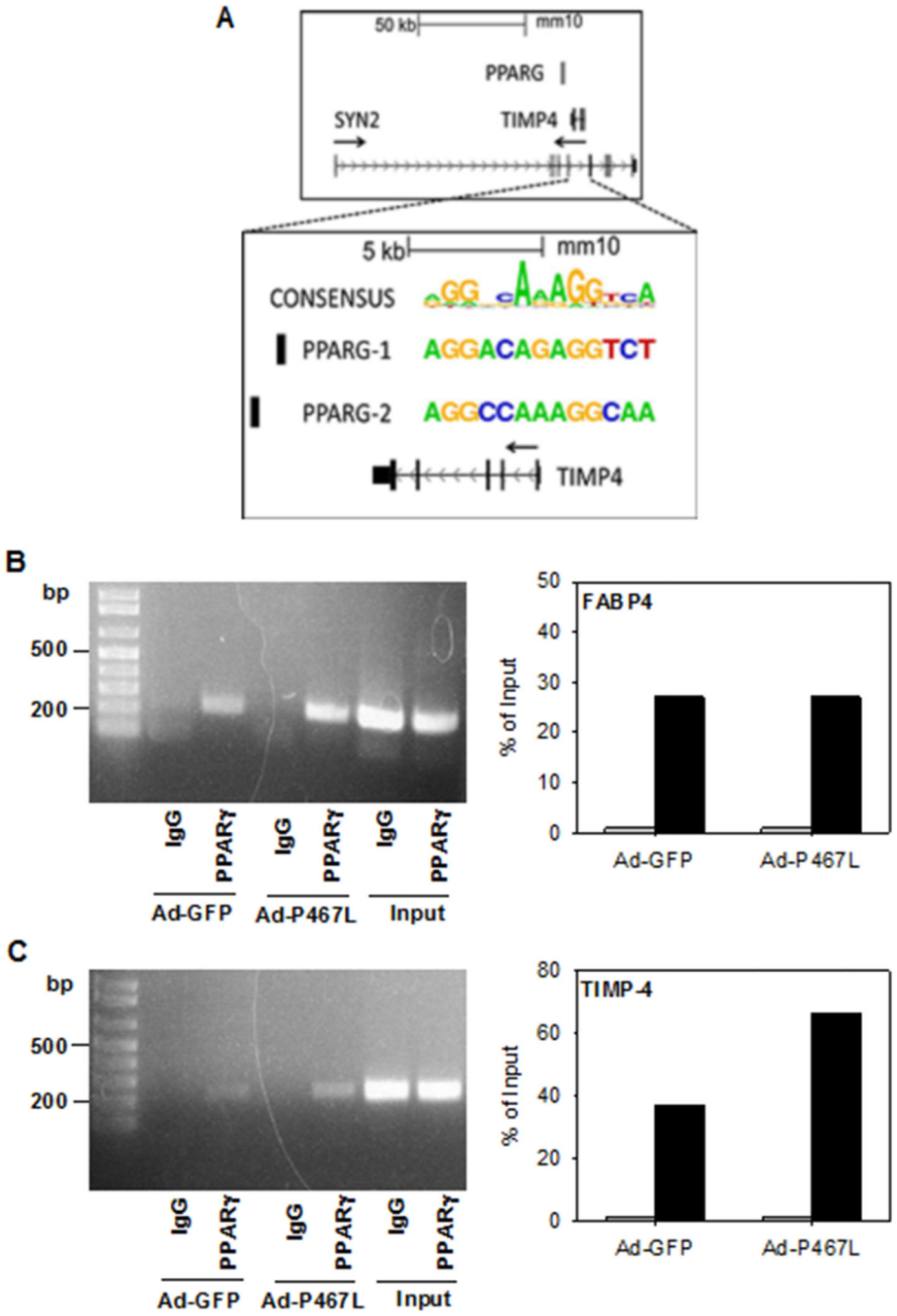

Figure 5. TIMP-4 is a novel PPARγ target

A) Top: Localization of the TIMP-4 gene and nearby PPARγ binding sites relative to the Synapsin 2 (SYN2) gene visualized using the UCSC genome browser. Arrows indicate the direction of transcription. Note that TIMP-4 is transcribed in reverse orientation (right to left). Bottom: Identification of PPREs near the TIMP-4 gene. PPRE sequences (PPARG-1 and PPARG-2) were identified downstream of the TIMP-4 gene in highly conserved regions shown to bind PPARγ in 3T3L1 adipocytes according to ChIP-Seq (Accession GSE13511). These sequences were identified using a position weighted matrix of known PPARγ binding

sites (Accession: MA0065.2) obtained from JASPAR (http://jaspar.genereg.net/), a database of experimentally verified transcription factor binding sites. The consensus of the position weighted matrix was visualized using sequence logos (created with WebLogo at http://weblogo.berkeley.edu/). B) ChIP analysis of FABP4 (B) and PPARγ (C) at PPARG-1 in RASMC transduced with either GFP or P467L PPARγ. Quantification determined by Q-RT-PCR (% of input, n=3).

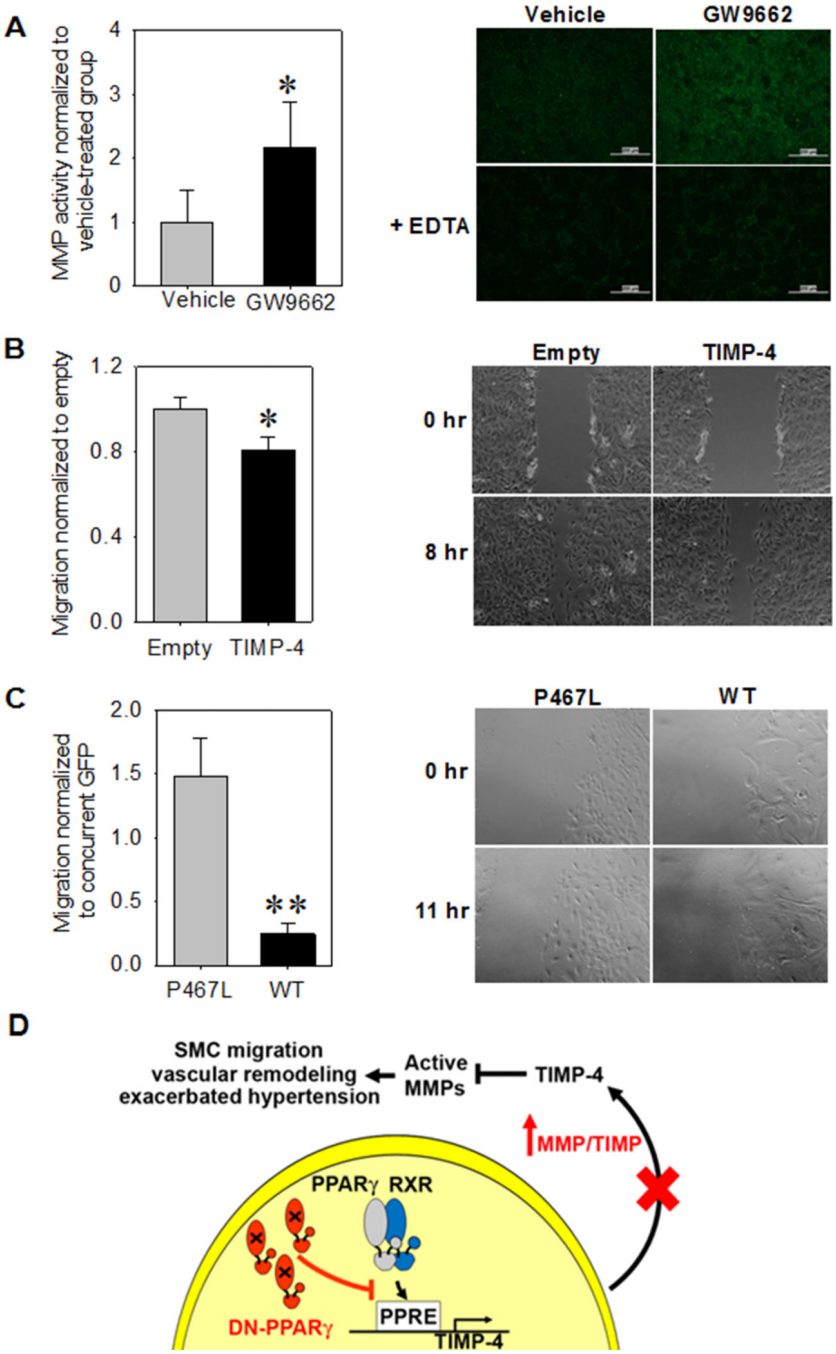

Figure 6. Increased MMP activity and SMC migration following PPARγ inhibition

A) Quantitative total MMP activity in RASMC after incubation with vehicle or 10 μM of GW9662 (Left) and the representation pictures (Right) (n=4); EDTA was used as a negative control. B) Quantitative migration assay from RASMC transfected with either empty vector or TIMP-4 plasmid (n=4). C) Scratch assay data from MSMC overexpressing P467L PPARγ compared to those with WT PPARγ transgene (n=3). *p<0.05 compared to vehicle or Empty; **p<0.05 compared to MSMC WT PPARγ. D) Model: In SMC, TIMP-4 expression is regulated by PPARγ through the direct binding with a PPRE at the TIMP-4 locus.

TIMP-4, an endogenous inhibitor of MMPs provides a tight control of SMC migration and vascular remodeling, which could subsequently impact the overall regulation of systemic BP. Impaired PPARγ activity via dominant negative (DN) mutation causes suppressed TIMP-4, increased MMP-9 and augmented SMC migration, which is associated with enhanced vascular remodeling and exacerbated salt-sensitive hypertension.