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Title Page

L-PGDS but not H-PGDS deletion causes hypertension and accelerates thrombogenesis in mice.

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Running title: L-PGDS causes hypertension and accelerates thrombogenesis.

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Abstract

Prostaglandin (PG) D_2 is formed by two distinct prostaglandin D synthases (PGDS): lipocalin-type PGDS (L-PGDS), which acts as a PGD_2 producing enzyme and as extracellular lipophilic transporter, and hematopoietic PGDS (H-PGDS), a sigma glutathione-S-transferase.

PGD_2 plays an important role in the maintenance of vascular function; however, the relative contribution of LPGDS and HPGDS dependent formation of PGD_2 in this setting is unknown.

To gain insight into the function played by these distinct PGDSs, we assessed systemic blood pressure (BP) and thrombogenesis in L-Pgds and H-Pgds KO mice. Deletion of L-Pgds depresses urinary PGD_2 metabolite (PGDM) by ~35% while deletion of H-Pgds does so by ~90%. Deletion of L-Pgds, but not H-Pgds, elevates BP and accelerates the thrombogenic occlusive response to a photochemical injury to the carotid artery. HQL-79, an H-PGDS inhibitor, further depresses PGDM in L-Pgds KO mice, but has no effect on BP or on the thrombogenic response. Gene expression profiling reveals that pathways relevant to vascular function are dysregulated in the aorta of L-Pgds KOs. These results indicate that the functional impact of L-Pgds deletion on vascular homeostasis may result from an autocrine effect of L-PGDS dependent PGD_2 on the vasculature and/or the L-PGDS function as lipophilic carrier protein.

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Introduction

Prostaglandin (PG) D_2 is synthesized in both the central nervous system (CNS) and the peripheral tissues (Ricciotti and FitzGerald, 2011). In the brain, PGD_2 is involved in the regulation of sleep and other neurological activities, including pain perception (Ricciotti and FitzGerald, 2011). In peripheral tissues, PGD_2 is produced mainly by mast cells and other cells, including platelets, macrophages, and lymphocytes, and there it plays a role in inflammatory and atopic diseases, although it might also exert an array of immunologically mediated anti-inflammatory functions (Ricciotti and FitzGerald, 2011).

PGD_2 is formed by the action of either lipocalin-like PGD synthase (L-PGDS) or hemopoietic PGD synthase (H-PGDS) (Urade Y and Hayaishi O, 2000). L-PGDS, known also as β trace protein, is a member of the lipocalin superfamily, a group of secretory proteins that bind and transport a variety of lipophilic molecules (Herlong JL and Scott TR, 2006). H-PGDS is a sigma class glutathione-S-transferase family member (Urade Y and Eguchi N, 2002). L-PGDS is expressed mainly in the CNS, retina, male and female genital organs, heart and vasculature, whereas H-PGDS is generally localized to the cytosol of immune and inflammatory cells. PGD_2 exerts it biological effects via two G protein-coupled receptors: the prostaglandin D receptor (DP) and the chemoattractant receptor–homologous molecule expressed on Th2 cells (CRTH2) (Hirai H et al., 2003; Kabashima K and Narumiya S, 2003; Sawyer N et al., 2002).

There is increasing evidence that PGDS and PGD_2 play a relevant role in the modulation of the vascular function (Ricciotti and FitzGerald, 2011). PGD_2 mediates vasodilation and increases vascular permeability (Braun M and Schror K, 1992), relaxation of vascular and non-vascular

smooth muscle (Narumiya S and Toda N, 1985; Hall IP, 2000) reduction ocular pressure (Goh Y et al., 1988), inhibition of platelet aggregation (Song WL et al., 2012) and chemotactic recruitment of inflammatory cells (Herlong JL and Scott TR, 2006).

L-PGDS expression is induced by laminar shear stress via activator protein 1 in vascular endothelial cells (Taba Y et al., 2000; Miyagi M et al., 2005) and it is expressed in synthetic smooth muscle cells in the intimal of atherosclerotic lesions and in coronary plaques of arteries with severe stenosis (Eguchi Y et al., 1997; Hirawa N et al., 2002). Patients with stable angina present significantly higher plasma level of L-PGDS in the cardiac vein than in the coronary artery and its concentration in the cardiac vein decreases immediately after angioplasty (Eguchi Y et al., 1997). Human serum L-PGDS level increases with the progression of atherosclerosis and it is an independent predictor of coronary severity (Inoue T, 2008). Moreover, elevated serum L-PGDS levels are associated with the presence of atrial fibrillation in hypertensive patients (Yalcin MU et al., 2016).

More recently it has been reported that serum level of L-PGDS may represent a biomarker of kidney function (White CA et al., 2015) pregnancy-induced hypertension (Duan B et al., 2016) and active lupus nephritis (Brunner HI et al., 2017).

In mice, L-PGDS deletion accelerates atherogenesis and induced glucose intolerance and obesity Ragolia L et al., 2005; Tanaka R et al., 2009). PGD_2 also plays a relevant role in the evolution of vascular inflammation. In the inflamed intima, PGD_2 is partly produced by H-PGDS-producing inflammatory cells that are chemotactically attracted to the vasculature (Herlong JL and Scott TR, 2006). PGD_2 has been shown to inhibit expression of pro-inflammatory genes, such as inducible nitric oxide synthase and plasminogen activator inhibitor-1 in vascular cells (Nagoshi H et al., 1998; Negoro H et al., 2002).

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The major objective of the present study was to elucidate the relative contributions of L-PGDS and H-PGDS derived PGD_2 to blood pressure (BP) homeostasis and thrombogenesis in mice. Our results indicate that H-PGDS makes a more substantial contribution than L-PGDS to PGD_2 biosynthesis, as reflected by the major urinary PGD_2 metabolite. Deletion of L-Pgds, but not of H-Pgds, predisposed both to higher BP and increased thrombogenesis.

Material and methods

Animals:

All animal protocols were approved by the University of Pennsylvania Institutional Animal Care and Use Committee. L-Pgds knock-out mice (L-Pgds KO, kindly provided by Dr. Yoshihiro Urade, Osaka, Japan), and H-Pgds knock-out mice (H-Pgds KO, kindly provided by Dr. Yoshihide Kanaoka, Boston, MA), all on a C57/BL6 background. Hence, wild type (WT) littermate controls were used in both sets of studies.

HQL-79, a H-PGDS inhibitor, was purchased from Tocris Bioscience.

WT and L-Pgds KO mice received HQL-79 (30 mg/kg/day in 0.5% methyl cellulose) by oral gavage for 10 days as previously reported (Aritake K et al., 2006; Mohri I et al., 2009).

Peritoneal macrophage culture

Peritoneal macrophages were collected 4 days after intraperitoneal injection of 0.5 mL of 10% thioglycollate (Sigma Chemicals, St Louis, Mo). Non-adherent cells were removed after 2 hours of incubation. Adherent cells were treated with 5 μg/mL of lipopolysaccharide (LPS, Sigma Chemicals, St. Louis, Mo) for 4 and 24 hours.

Mass spectrometric analysis of prostanoids and their metabolites

Prostanoids or their metabolites were measured by mass spectrometry as described previously (Song WL, et al., 2007). Briefly, macrophage production of PGD_2 was determined by quantification of PGD_2 in cell culture supernatants and normalized with total protein content. Systemic production of PGE_2, PGD_2, TxA_2, and prostacyclin (PGI_2) were determined by quantification of their major urinary metabolites (7-hydroxy-5,11-diketotetranorprostane-1,16-dioic acid [PGE-M], 11,15-dioxo-9α-hydroxy-, 2,3,4,5-tetranorprostan-1,20-dioic acid [tetranor PGD-M], 2,3-dinor TxB_2 [Tx-M], and 2,3-dinor 6-keto $PGF_{1\alpha}$ [PGI-M], respectively) in 24-hour collections and normalized with creatinine.

Blood pressure measurement by tail-cuff

Resting systolic blood pressure was measured in conscious WT, L-Pgds KOs and H-Pgds KOs, 8-12 weeks old mice, using a computerized noninvasive tail cuff system (Visitech Systems Inc.). Mice were trained to adapt to the system for 14 days. After that, blood pressure was recorded daily for 3 consecutive days at the same time of the day and in the same way. Data were collected and analyzed using updated BP-2000 software (Visitech Systems Inc.).

Photochemical injury of the carotid artery

L-Pgds KO, H-Pgds KO mice and their littermate WT control mice underwent photochemically induced vascular injury in the carotid artery as previously reported (Cheng Y et al., 2006). Briefly, in anesthetized (sodium pentobarbital, 80 mg/kg) mice 12–16 weeks of age, the left common carotid artery was isolated, and a Doppler flow probe (model 0.5 VB; Transonic Systems Inc.) was applied. The probe was connected to a flowmeter (model T105; Transonic

Systems Inc.) and interpreted with a computerized data acquisition program (PowerLab; AD Instruments). Rose Bengal (Fisher Scientific International) was injected into the jugular vein in a volume of 0.12 ml in a final concentration of 50 mg/kg. Vascular injury was induced by applying 1.5 mW green light laser (540 nm) (Melles Griot) at a distance of 5 cm from the desired site on the carotid artery. Blood flow was monitored for 120 minutes or until stable occlusion occurred. Stable occlusion was defined as a blood flow of 0 ml/min for 3 minutes. Mice that did not occlude within the 120-minute time course were excluded from the experiment. Complete and 50% occlusion time were determined.

Microarray analysis

Thoracic aortas were harvested from male WT and L-Pgds KO mice, 8-12 weeks old. Total RNA was prepared using TRIzol reagent (Invitrogen) and RNeasy columns (Qiagen, Valencia, CA). Samples were prepared in one batch using the Nugen sample preparation protocol and hybridized to Affymetrix MOE430 v2. Data from the CEL files were summarized and normalized using print-tip loess algorithm from the Bioconductor array package for R.6 Patterns of Gene Expression (PaGE) software, which uses the False Discovery Rate (FDR) method, was used to identify statistically significant differentially expressed gene (DEG)s, as previously described (Grant GR et al., 2005). The FDR was set at 10% for all differential gene expression analyses, so that 90% the predicted genes are expected to be true positives. The expression data were clustered (hierarchical method) and visualized using a modified version of the heatmap{stats} function in R.

Ingenuity Pathways Analysis (Ingenuity Systems, www.ingenuity.com) was employed to identify relevant canonical pathways enriched by DEGs between WT and L-Pgds KO mice.

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Gene Ontology (GO) enrichment analysis was employed to identify enriched GO biological functions (http://geneontology.org/page/go-enrichment-analysis).

Microarray data are available in the GEO database (http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE77642.

Real-Time PCR analysis

Total RNAs isolated from aortas, brain, adrenal gland and heart (as described above) were reverse-transcribed into cDNA by Taqman Reverse Transcription Reagents (Applied Biosystems, Foster City, CA). TaqMan Gene Expression Assays (Applied Biosystems) for Phenylethanolamine N-methyltransferase (Pnmt, Mm00476993_m1), L-Pgds(Mm01330513_m1), H-Pgds (Mm00479848), Dp1 (Mm00436050_m1) and Dp2 (Mm01223054_m1)were performed on an ABI Prism 7900 Sequence Detection System (Applied Biosystems). Results were normalized with 18s rRNA (Hs99999901_s1).

Statistics

Unless otherwise indicated, between-group comparison was performed using the non-parametric Mann–Whitney test or non-parametric one-way ANOVA. All the comparisons were made between animals of the same age and gender on the same genetic background. A significance threshold of 0.05 was used and it was indicated by a single asterisk. Significance of <0.01 is indicated by double asterisks on the graphs and significance <0.001 is indicated by triple asterisks. All data are presented as mean ± SEM unless otherwise stated.

Results

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Differential impact of L-Pgds and H-Pgds deletion on urinary PGD_2 metabolite

Deletion of L-Pgds depressed PGDM by ~30% in both genders when compared to wild type littermate controls (by 34.5% in males, $P < 0.05$, n = 14-15; by 35.3 % in females, $P < 0.05$, n = 11, Fig.1A). Deletion of H-Pgds had a more pronounced effect, depressing urinary PGDM by ~90% (by 91.3% in males, $P < 0.0001$ n = 12; by 87.8% in females, $P < 0.0001$, n = 11-14; Fig. 1B). There was no evidence of systemic re-diversion of the PGH_2 substrate as urinary PGEM, TXM and PGIM were unaltered in both mutants (Suppl. Fig.1).

Deletion of H-Pgds, but not L-Pgds, suppresses PGD_2 biosynthesis by macrophages

H-Pgds was the primary source of PGD_2 formation under basal conditions and after LPS stimulated peritoneal macrophages (Suppl. Fig. 2). PGD_2 production increased after 24 hrs of LPS stimulation (Suppl. Fig. 2A and 2B). Deletion of H-Pgds almost completely suppressed PGD_2 production by macrophages (by 80 % at baseline, $P < 0.0001$, n = 9-15; by 98% at 24 hrs of LPS stimulation, $P < 0.0001$, n = 10-22, Suppl. Fig. 2A). In contrast, deletion of L-Pgds had no impact on PGD_2 production by macrophages (Suppl. Fig. 2B).

Deletion of L-Pgds, but not H-Pgds, elevates blood pressure

Systolic blood pressure was measured by tail-cuff in both male and female L-Pgds and H-Pgds KOs and their littermate controls. L-Pgds deletion caused a statistically significant increase in systolic blood pressure in both females and males (117.5 ± 2.7 *vs.* 126.3 ± 2.5 mmHg in females, $P < 0.05$, n = 9-11; 119.3 ± 2.4 *vs.* 131.2 ± 4.2 mmHg in males, $P < 0.05$, n = 11-18; Fig. 2 A and B). H-Pgds deletion did not cause any change in blood pressure in either gender (116.9 ± 2.8 *vs.*

112.9± 2.8 mmHg in females, n = 10-11; 111.7 ± 5.4 *vs.* 113.2 ± 7.3 mmHg in males, n = 5-8; Fig. 2 C and D).

Deletion of L-Pgds, but not H-Pgds, accelerates the thrombogenic occlusive response to a photochemical injury of the carotid artery

The time to complete common carotid artery occlusion after photochemical injury was reduced by L-Pgds deletion by approximately 45% in females and 30% in males (68.6 ± 7.1 vs. 38.2 ± 7.8 minutes in females, $P < 0.01$, n = 10-11; 59.0 ± 4.0 vs. 41.7 ± 2.7 minutes in males, $P < 0.001$, n = 11-16; Fig. 3 A and B). The time to complete common carotid artery occlusion in either gender was unaltered by H-Pgds deletion (Fig. 3 C and D).

HQL-79, a selective H-PGDS inhibitor, has no effect on blood pressure or thrombogenic response

HQL-79, a selective inhibitor of H-PGDS (Aritake K et al., 2006; Mohri I et al., 2009), caused a further reduction of PGDM level by approximately 30% in L-Pgds KOs (8.9 ± 0.9 *vs.* 6.0 ± 0.7 ng/mg creatinine, WT *vs*. L-Pgds KO+ vehicle, $P < 0.05$, n = 15-17; 6.0 ± 0.7 vs. 4.0 ± 0.6 ng/mg creatinine, L-Pgds KO+vehicle *vs.* L-Pgds KO+HQL-79, $P < 0.05$, n = 17; Fig. 4 A). The additional reduction of PGDM caused by HQL-79 administration in L-Pgds KO mice did not have any effect on blood pressure (123.6 ± 2.4 *vs.* 123.0 ± 2.5 mmHg, L-Pgds KO+vehicle *vs.* L-Pgds KO+HQL-79, n=18; Fig. 4 B) and on the thrombogenic response (71.5 ± 10.8 *vs.* 63.0 ± 6.2 minutes, L-Pgds KO+vehicle *vs.* L-Pgds KO+HQL-79, n=6-9; Fig. 4 C).

L-Pgds deletion causes dysregulation of genes associated with hypertension and thrombosis

To gain insight into potential mechanisms by which L-Pgds deletion contributes to the hypertensive and pro-thrombotic phenotype, the transcriptome of aorta samples from L-Pgds KO was compared to those from WT mice with microarrays. Deletion of L-Pgds have no effects on expression of H-Pgds, PGD_2 receptors Ptgdr (Dp1) and Ptgdr2 (Dp2) (Suppl Fig 3, 4). The analysis revealed a differential gene expression profile in the aorta from L-Pgds KO compared to control mice. In particular, a total of 454 probes (corresponding to 391 unique genes) were differentially expressed in the aorta of L-Pgds KO mice (q<0.1; Table 1), of which 186 were up-regulated and 268 down-regulated as compared to WTs (Fig. 5). As expected, the samples clustered according to their genotype and the most down-regulated gene in the aorta of L-Pgds KO mice was Ptgds (fold change of 40), which encodes the L-Pgds enzyme.

Among the genes up-regulated in L-Pgds KO mice, we identified Pnmt (fold change of 1.5), a N-methyltransferase that methylates norepinephrine (NE) to form epinephrine (E). We confirmed an increased expression of Pnmt in the aorta, brain and adrenal gland but not in the heart of L-Pgds KO mice by rt-PCR (Fig. 6). Pnmt`s mRNA level was reported to be positively correlated with systemic blood pressure (Reja V et al., 2002) and, indeed both male and females L-Pgds KO showed an increased systolic blood pressure (Fig. 2 A-B).

It has been reported that L-Pgds KO mice show features of the metabolic syndrome that correlates with hypothalamic-pituitary-adrenal (HPA) hyperactivity (Evans JF et al., 2013). Indeed, corticotropin releasing hormone receptor 2 (Crhr2) was upregulated 1.6 fold in the aorta of L-Pgds KO mice.

In addition, significant changes in the expression of a number of genes involved in glucose metabolism and insulin signaling were identified in the aorta of L-Pgds KO mice, including the up-regulation of nicotamide nucleotide transhydrogenase (Nnt, fold change of 2.5) and the down-

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regulation of insulin receptor (Insr, fold change of 1.5), insulin degrading enzyme (Ide, fold change 1.4), lipin 1 (Lpin1, fold change of 1.6), Acyl-CoA synthetase medium-chain family member 3 (Acsm3, fold change of 1.6), acyl-CoA synthetase long-chain family member 4 (Acsl4, fold change of 1.8) and glycogen synthase-1 (Gys1, fold change of 1.3).

Changes in the expression of these genes are consistent with the insulin resistant phenotype observed in the L-Pgds KO (Ragolia L et al., 2005).

It is well established that insulin resistance contributes to endothelial dysfunction and a consequent predisposition to thrombosis in diabetics. Increased oxidative stress may also contribute to this phenotype (Ceriello A and Motz E, 2004). Indeed, several aortic genes related to the oxidative stress response, such as heme oxygenase 1 (Hmox1, fold change of 1.4), glutathione S-transferase mu (Gstm2, fold change of 1.2) and thioredoxin interacting protein (Txnip, fold change of 1.5) were dysregulated in L-Pgds KO mice.

Finally, Ingenuity Pathways analysis revealed that the top canonical pathways enriched by genes differentially regulated between WT and L-Pgds KO mice are related to the cellular stress response like “Protein Ubiquitination Pathway”, “Unfolded protein response”, “NRF2 mediated oxidative stress response” and “Production of nitric Oxide and reactive oxygen species” (Fig. 7).

Interestingly, GO Enrichment Analysis revealed that all the top10 molecular functions enriched by genes differentially regulated between WT and L-Pgds KO mice are related to protein binding function (Fig. 8) and about 6% of DEGs are transporters and most of them are members of the solute carrier group of membrane transport proteins (solute carrier family 4, 7, 11, 12, 22, 25, 26, 39, 43, 50, Table 1).

Gene expression profiles of L-Pgds and H-Pgds are summarized in Suppl. Table 1

Discussion

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Both human and mouse studies indicate an important role of PGD_2 in the vasculature.

In this study, we demonstrated that the systemic biosynthesis of PGD_2 is generated mainly through H-Pgds (about 90%) and only partially through L-Pgds (Fig 1). However, deletion of L-Pgds, but not H-Pgds, elevated systemic blood pressure (Fig. 2) and accelerated the thrombogenic occlusive response to a photochemical injury to the carotid artery in mice (Fig 3). Thus, the effect of L-Pgds deletion on these cardiovascular phenotypes was not directly or quantitatively related to the suppression of systemic PGD_2 biosynthesis. In fact, HQL-79, an H-PGDS inhibitor, further depresses urinary PGDM in L-Pgds KO mice, but has no incremental effect on blood pressure level or the thrombogenic response in mice (Fig 4). Thus, although it is known that PGD_2 can induce relaxation of vascular and non-vascular smooth muscle cells (Braun M and Schror K, 1992; Narumiya S and Toda N, 1985) and inhibit platelet activation and aggregation by increasing adenylate cyclase activity (Bushfield M et al., 1985) we failed to observe any effect of H-Pgds deletion, the major contributor of systemic production of PGD_2, on blood pressure or thrombosis.

Previously, L-PGDS had been linked to blood pressure homeostasis: both serum and urinary levels of L-PGDS are much higher in patients with hypertension than those in normotensive subjects (Hirawa N et al., 2002). Moreover, increased platelet P-selectin expression, an index of platelet activation, is negatively correlated with a decrease in serum L-PGDS after percutaneous transluminal coronary angioplasty in the coronary sinus (Inoue T et al., 2001).

L-PGDS, a member of the lipocalin superfamily, functions as a PGD_2-producing enzyme and as a lipophilic carrier protein in the extracellular environment, binding several molecules, such as retinoids, thyroid hormones, bile pigments, fatty acids, hemoglobin and cannabinoid metabolites (Herlong JL and Scott TR, 2006; Orenes-Piñero E et al., 2013, Elmes MW et al., 2018; Zhou Y

et al., 2010). Recently, it has also been reported that L-PGDS can bind hydrophilic ligands like NADPH (Qin S et al., 2015).

The role of L-PGDS as lipophilic carrier protein has already been linked to the insulin resistant and atherosclerotic phenotypes observed in L-Pgds KO mice (Ragolia L et al., 2005).

The role of L-PGDS as a retinoid transporter might be involved in this process since a link exists between the retinoid X receptor and adipogenesis (Ragolia L et al., 2005).

Here, an unbiased analysis of the aortic transcriptomic profile revealed that genes relevant to blood pressure and thrombosis are dysregulated in L-Pgds KO mice compared to the controls (Table 1). Pnmt was one of genes most highly over expressed in the aorta (and other tissues relevant to catecholamine biosynthesis, such as brain and adrenal glands) of L-Pgds deficient mice (Fig 6).

Previously, it has been reported that deletion of L-Pgds accelerates glucose intolerance and insulin resistance (Ragolia L et al., 2005). Indeed, several genes involved in glucose metabolism and insulin signaling were differentially regulated in the aorta of L-Pgds KO mice compared to wild type mice, including up-regulation of Nnt and down-regulation of Insr, Lipin 1, Acsm3, Acsl4 and Gys1. Moreover, Ingenuity Pathway Analysis revealed that pathways related to cellular stress response like “Protein Ubiquitination Pathway”, “Unfolded protein response”, “NRF2 mediated oxidative stress response” and “Production of nitric Oxide and reactive oxygen species” were enriched in those genes differentially expressed in WT and L-Pgds KO mice. These data are consistent with the hypothesis that insulin resistance in L-Pgds KO mice might result in oxidative stress in the vasculature, resulting in the hypertension and exaggerated thrombogenesis that we observed.

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Although suppression of PGD_2 biosynthesis by deletion of H-Pgds has no resultant cardiovascular phenotype, this might be expected given the absence of expression of its transcript in vascular cells. While the phenotypes consequent to deletion of L-Pgds may reflect its actions independent of PGD_2, it is possible that L-Pgds confers PGD_2 dependent cardioprotection via an autocrine effect in the vasculature. Off target effects, particularly mediated via its transporter function, may also confer cardioprotection, alone or in conjunction with an impact on PGD_2. Indeed, 6% of the 391 unique genes differentially expressed in the aorta of L-Pgds deficient mice compared to control mice are transporters and most of them are members of the solute carrier group of membrane transport proteins (Fig. 9, Table 1). Moreover, the GO Enrichment Analysis revealed that all the top molecular functions enriched by genes differentially regulated between WT and L-Pgds KO mice are related to binding functions (Fig. 8).

In conclusion, these results indicate that the functional impact of L-Pgds deletion on vascular homeostasis may result from an autocrine effect of L-PGDS dependent PGD_2 on the vasculature and/or the L-PGDS function as lipophilic carrier protein.

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Authorship Contributions

Participated in research design: Song, Ricciotti, FitzGerald.

Conducted experiments: Song, Ricciotti, Liang.

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Contributed new reagents or analytic tools: Song, Ricciotti.

Performed data analysis: Song, Ricciotti, Grosser, Grant.

Wrote or contributed to the writing of the manuscript: Song, Ricciotti, FitzGerald.

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Footnotes

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Figure Legends

Figure 1. Impact of L-Pgds and H-Pgds deletion on urinary PGD_2 metabolite.

A) Urinary PGD_2 metabolite (PGDM) measured in wild-type (WT) and L-Pgds KO male (blue bars) and female (red bars) mice. B) PGDM measured in WT and H-Pgds KO male (blue bars) and female (red bars) mice. Data in A and B are mean±SEM, n=11-15; *p<0.05; ****p<0.0001.

Figure 2. Effect L-Pgds deletion on systolic blood pressure.

A) Systolic blood pressure (BP) measured in WT and L-Pgds KO female mice. B) BP measured in WT and L-Pgds KO male mice. C) BP measured in WT and H-Pgds KO female mice. D) BP measured in WT and H-Pgds KO male mice. Data are mean±SEM, n=5-18; *p<0.05.

Figure 3. Effect L-Pgds deletion on photochemical induced thrombogenesis in carotid artery.

A) Time to complete common carotid artery occlusion after photochemical injury (Thrombosis time) measured in WT and L-Pgds KO female mice. B) Thrombosis time measured in WT and L-Pgds KO male mice. C) Thrombosis time measured in WT and H-Pgds KO female mice. D) Thrombosis time measured in WT and H-Pgds KO male mice. Data are mean±SEM, n=10-16; **p<0.01.

Figure 4. Effect of HQL-79, a selective H-PGDS inhibitor, on urinary PGD_2 metabolite, systolic blood pressure and thrombogenesis in carotid artery in L-Pgds KO mice.

A) Urinary PGD_2 metabolite (PGDM) measured in wild-type (WT) and in L-Pgds KO male mice treated with vehicle or HQL-79. B) Systolic blood pressure (BP) measured in L-Pgds KO male

mice treated with vehicle or HQL-79. C) Time to complete common carotid artery occlusion after photochemical injury (Thrombosis time) measured in L-Pgds KO male mice treated with vehicle or HQL-79. Data are mean±SEM, n=6-18; *p<0.05; ***p<0.001.

Figure 5. Effect of L-Pgds deletion in mouse aorta.

Heatmap representation of 391 genes differentially expressed (q<0.1) between WT and L-Pgds KO male mice in aorta.

Figure 6. Effect of L-Pgds deletion on Pnmt expression.

Expression of Pnmt in aorta (A), heart (B), brain (C) and adrenal gland (D) in WT and L-Pgds male KO mice measured by rt-pcr. Data are mean±SEM, n=5; *p<0.05.

Figure 7. Ingenuity Pathway Analysis.

Top 20 canonical pathway enriched by genes differentially expressed (q<0.1) between WT and L-Pgds KO mice in aorta.

Figure 8. GO enrichment analysis.

Top 10 GO molecular function enriched by genes differentially expressed (q<0.1) between WT and L-Pgds KO male mice in aorta.

Fig. 1. Impact of L-Pgds and H-Pgds deletion on PGDM

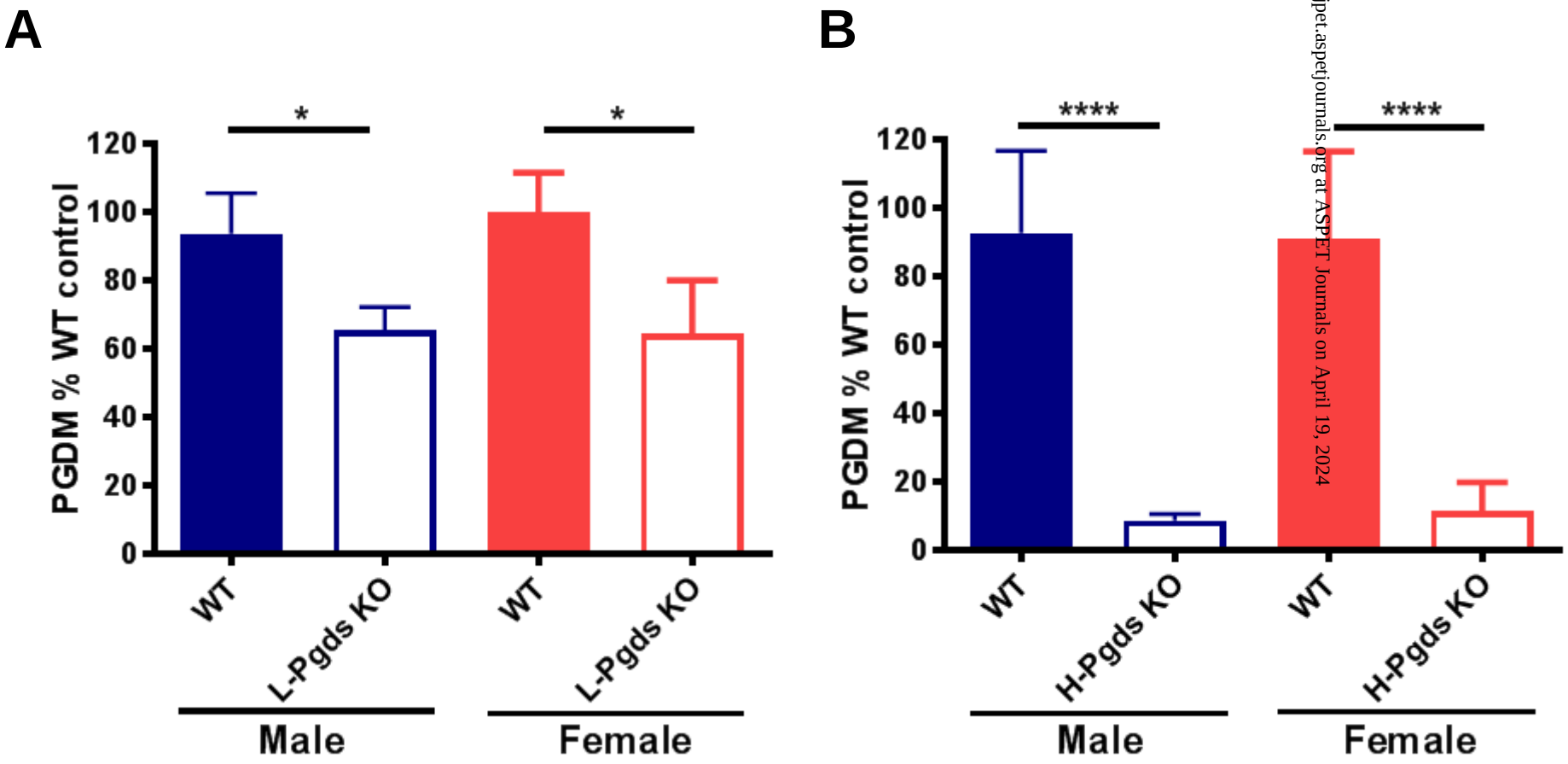


Fig. 2 L-Pgds deletion elevates systolic blood pressure

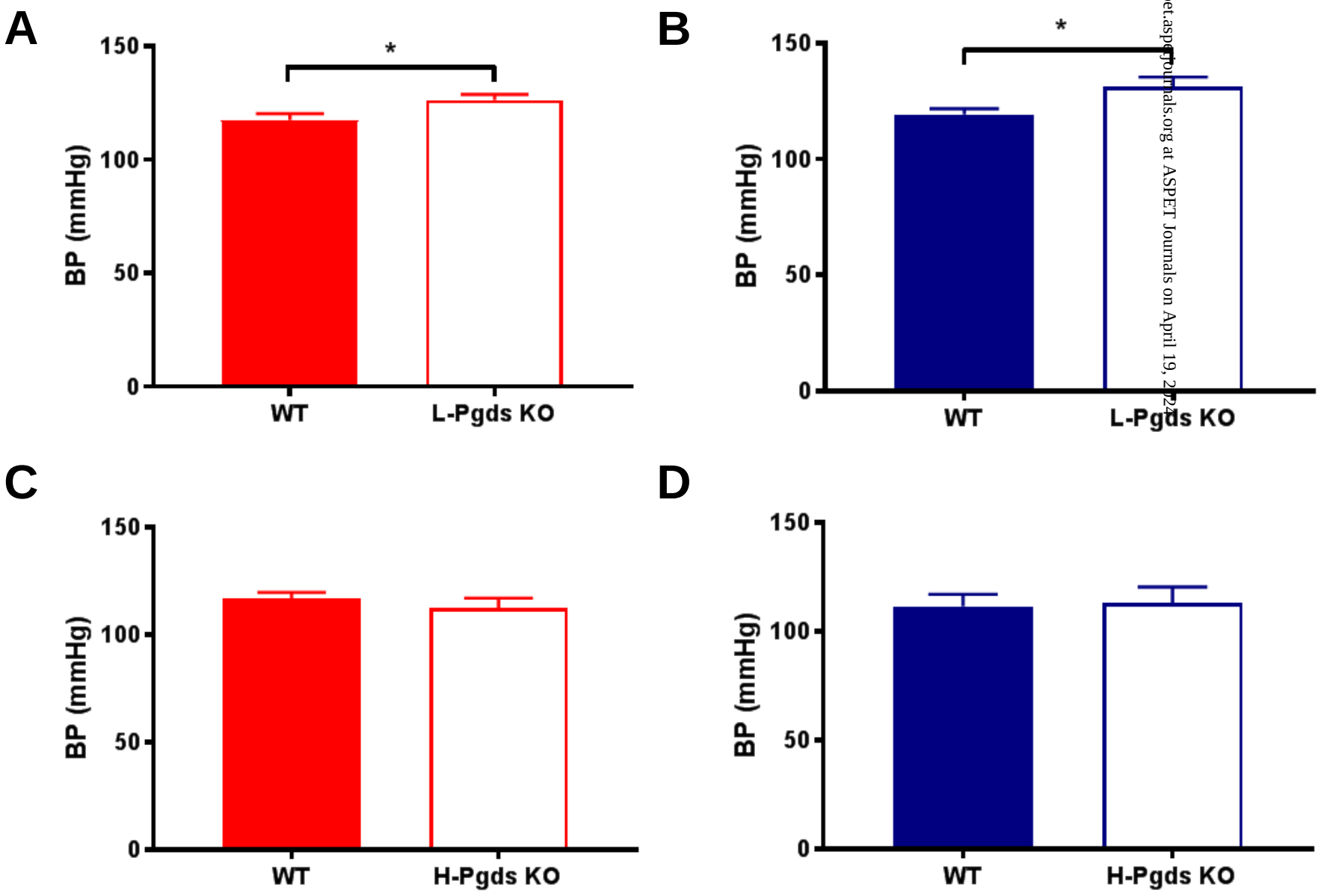


Fig. 3 L-Pgds deletion accelerates photochemical induced thrombogenesis in carotid artery

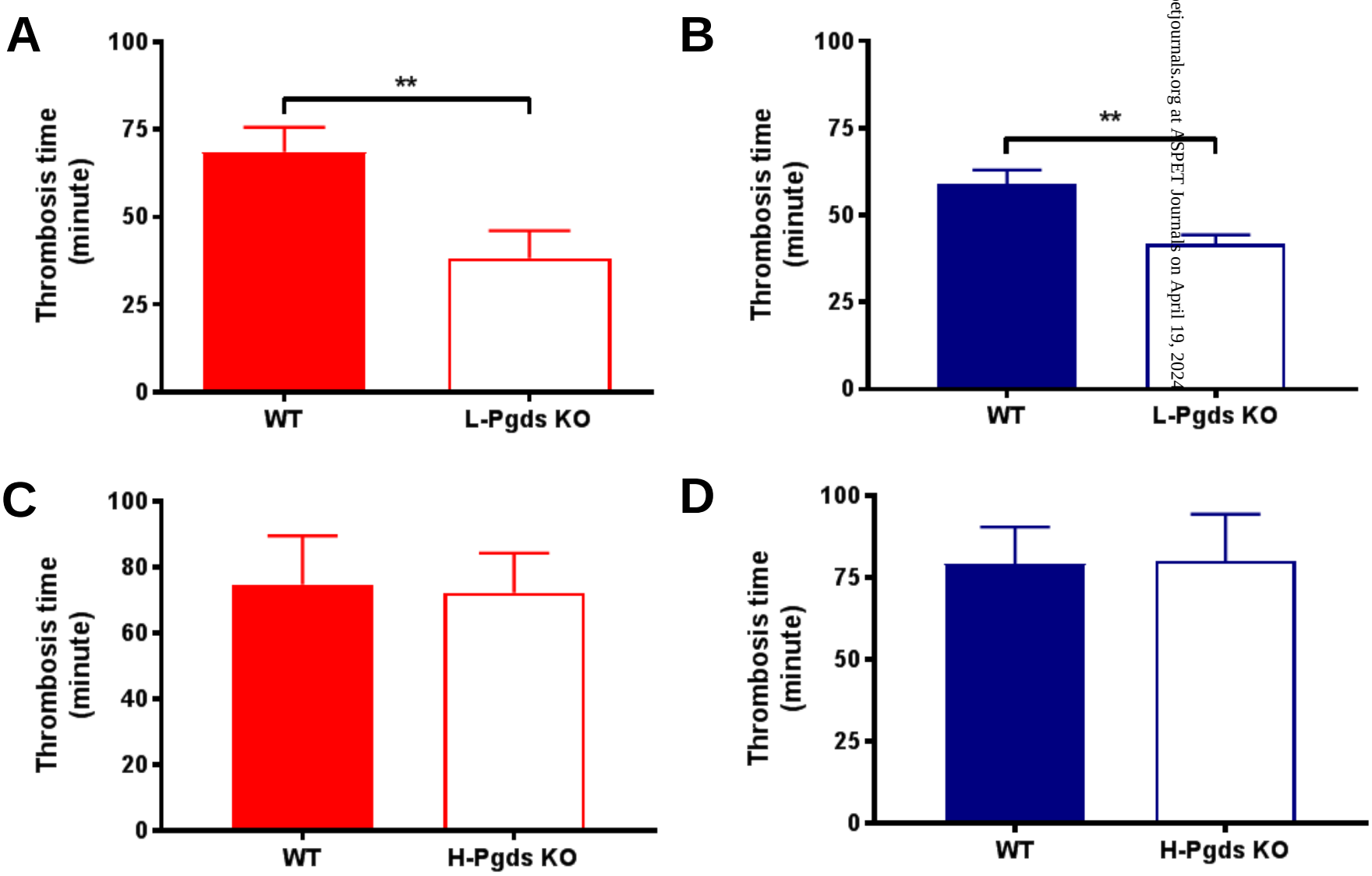
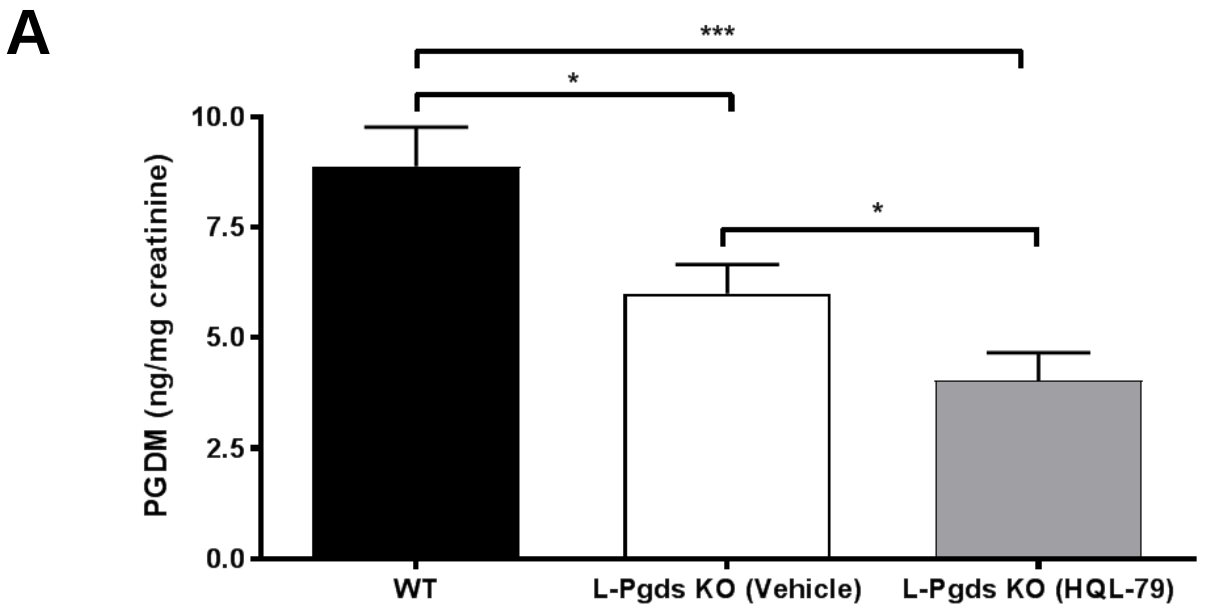
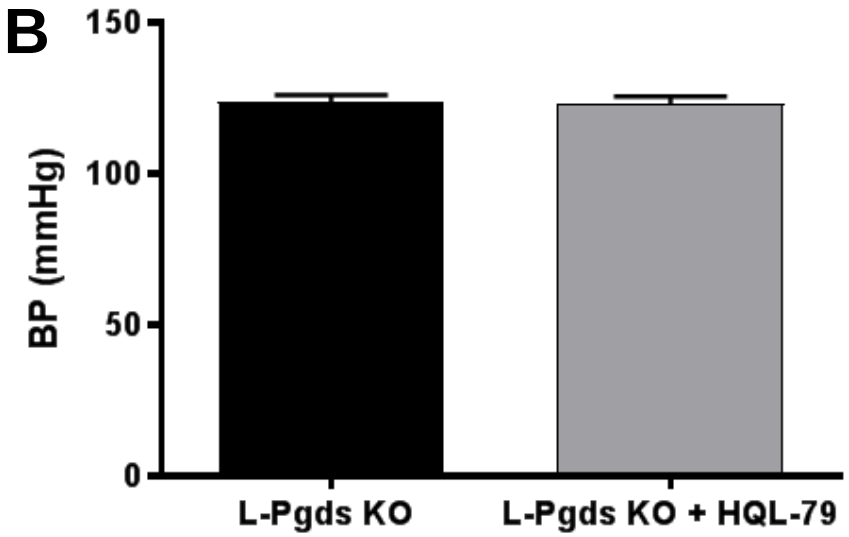


Fig. 4 HQL-79, a H-PGDS inhibitor, does not impact blood pressure and thrombogenic response





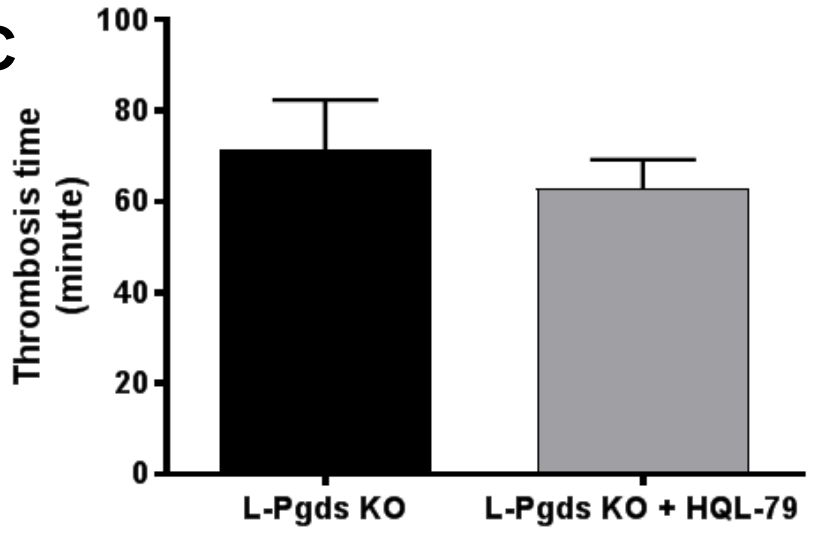


Fig. 5 Effect of L-Pgds deletion in mouse aorta.

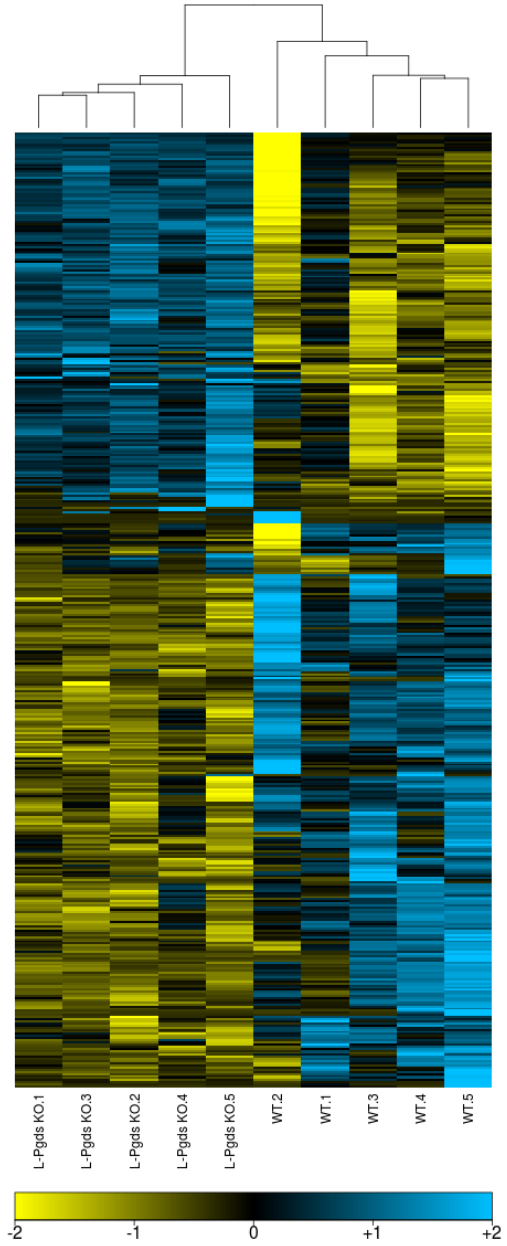


Fig. 6 Effect of L-Pgds deletion on Pnmt expression profile in different tissues

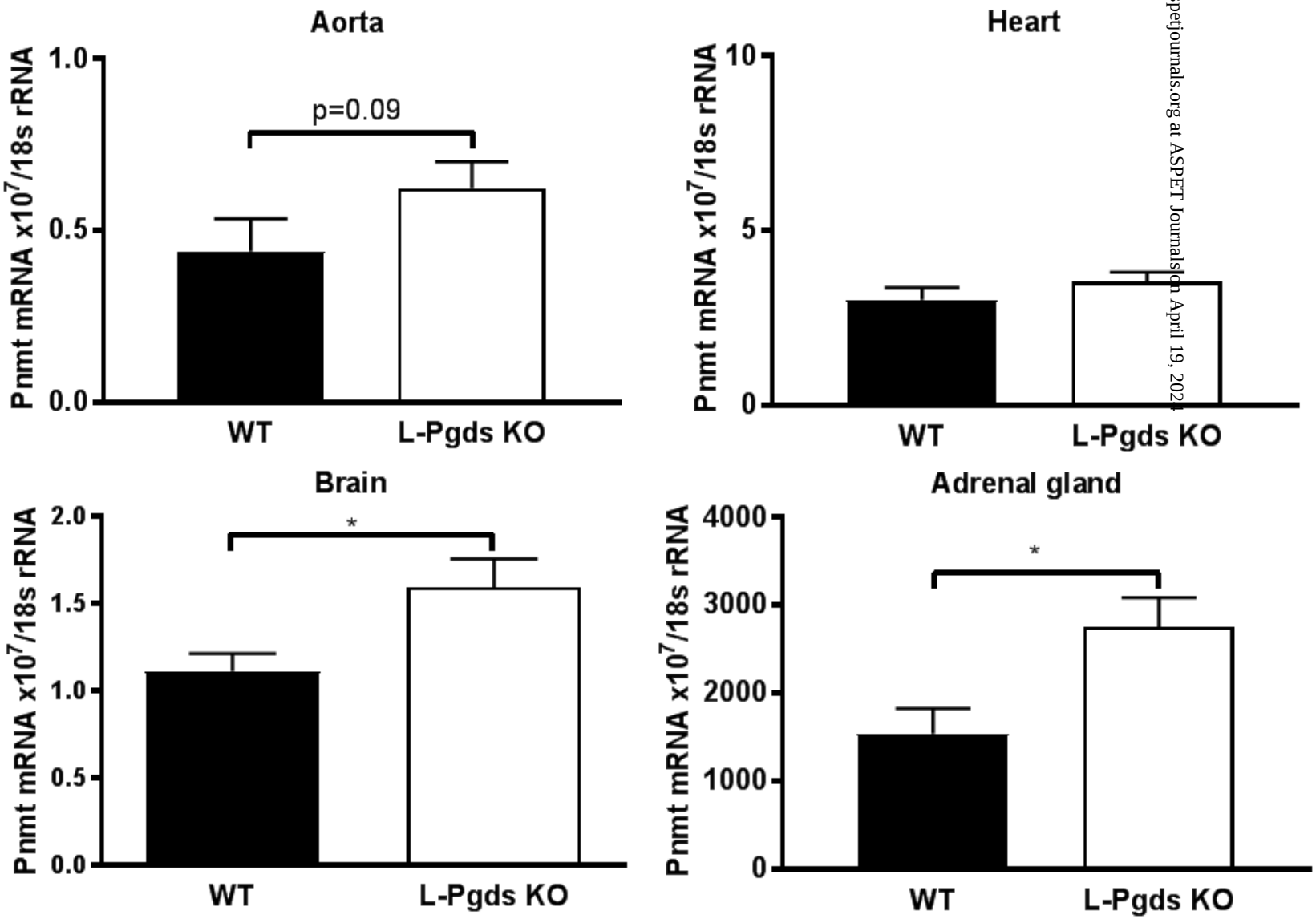


Fig. 7 Top 20 canonical pathway enriched by genes differentially expressed between WT and L-Pgds KO mice

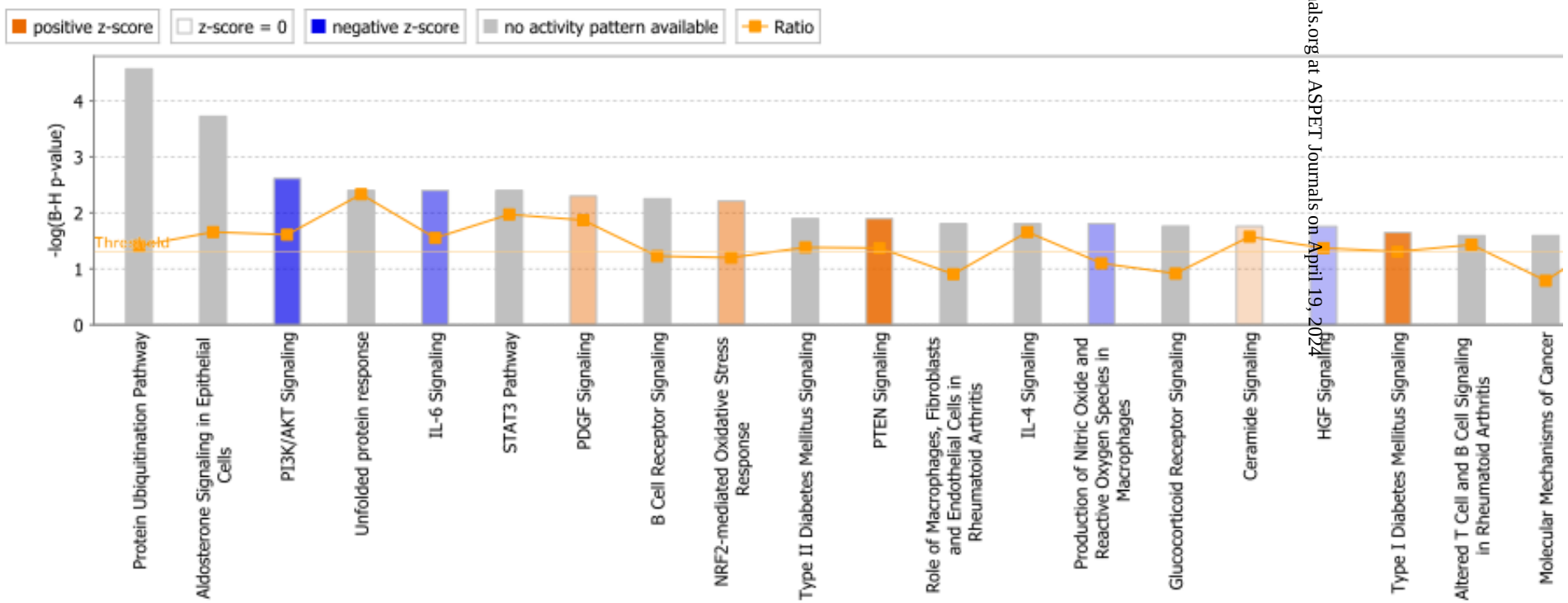


Fig. 8 Top 10 GO molecular function enriched by genes differentially expressed between WT and L-Pgds KO mice

GO molecular function complete	Fold Enrichment	P-value
binding (GO:0005488)	1.54	2.85E-31
protein binding (GO:0005515)	1.87	1.07E-29
heterocyclic compound binding (GO:1901363)	1.78	1.18E-11
organic cyclic compound binding (GO:0097159)	1.76	2.04E-11
enzyme binding (GO:0019899)	2.39	1.10E-08
nucleoside phosphate binding (GO:1901265)	2.18	4.23E-08
nucleotide binding (GO:0000166)	2.18	4.23E-08
carbohydrate derivative binding (GO:0097367)	2.2	4.60E-08
small molecule binding (GO:0036094)	2.04	1.70E-07
receptor binding (GO:0005102)	2.45	2.46E-07