

**ORIGINAL RESEARCH**

# Niacin Attenuates Pulmonary Hypertension Through H-PGDS in Macrophages

Daile Jia,\* Peiyuan Bai,\* Naifu Wan,\* Jiao Liu,\* Qian Zhu, Yuhu He, Guilin Chen, Jing Wang<sup>ID</sup>, Han Chen, Chen Wang, Ankang Lyu, Michael Lazarus, Yunchao Su, Yoshihiro Urade, Ying Yu, Jian Zhang, Yujun Shen<sup>ID</sup>

**RATIONALE:** Pulmonary arterial hypertension (PAH) is characterized by progressive pulmonary vascular remodeling, accompanied by varying degrees of perivascular inflammation. Niacin, a commonly used lipid-lowering drug, possesses vasodilating and proresolution effects by promoting the release of prostaglandin D<sub>2</sub> (PGD<sub>2</sub>). However, whether or not niacin confers protection against PAH pathogenesis is still unknown.

**OBJECTIVE:** This study aimed to determine whether or not niacin attenuates the development of PAH and, if so, to elucidate the molecular mechanisms underlying its effects.

**METHODS AND RESULTS:** Vascular endothelial growth factor receptor inhibitor SU5416 and hypoxic exposure were used to induce pulmonary hypertension (PH) in rodents. We found that niacin attenuated the development of this hypoxia/SU5416-induced PH in mice and suppressed progression of monocrotaline-induced and hypoxia/SU5416-induced PH in rats through the reduction of pulmonary artery remodeling. Niacin boosted PGD<sub>2</sub> generation in lung tissue, mainly through H-PGDS (hematopoietic PGD<sub>2</sub> synthases). Deletion of H-PGDS, but not lipocalin-type PGDS, exacerbated the hypoxia/SU5416-induced PH in mice and abolished the protective effects of niacin against PAH. Moreover, H-PGDS was expressed dominantly in infiltrated macrophages in lungs of PH mice and patients with idiopathic PAH. Macrophage-specific deletion of H-PGDS markedly decreased PGD<sub>2</sub> generation in lungs, aggravated hypoxia/SU5416-induced PH in mice, and attenuated the therapeutic effect of niacin on PAH.

**CONCLUSIONS:** Niacin treatment ameliorates the progression of PAH through the suppression of vascular remodeling by stimulating H-PGDS-derived PGD<sub>2</sub> release from macrophages.

**GRAPHIC ABSTRACT:** A graphic abstract is available for this article.

**Key Words:** macrophage ■ niacin ■ pulmonary arterial hypertension ■ pulmonary artery remodeling

---

## Meet the First Author, see p 1219

---

Pulmonary arterial hypertension (PAH) is a rare and fatal cardiopulmonary disorder characterized by progressive pulmonary arterial remodeling, which results in increased pulmonary vascular resistance, and eventually, in right ventricular failure.<sup>1</sup> The imbalances between potent vasodilators and vasoconstrictors associated with endothelial dysfunction, and the enhanced proliferation and hypertrophy of pulmonary arterial vascular smooth

muscle cells (PASMCs), are the key pathophysiological components of pulmonary vascular remodeling in PAH.<sup>2</sup> Recently, inflammation and autoimmunity have been recognized as critical contributors to this pulmonary vascular remodeling.<sup>3</sup> Pathological specimens from patients with PAH reveal an accumulation of perivascular inflammatory cells, including macrophages, dendritic cells, T and B lymphocytes, and mast cells.<sup>4</sup> Secreted factors produced

Correspondence to: Yujun Shen, PhD, Department of Pharmacology, School of Basic Medical Sciences, Tianjin Medical University, 22 Qixiangtai Rd, Heping District, Tianjin 300070, China, Email yujun\_shen@tmu.edu.cn; or Jian Zhang, PhD, Department of Pharmacology, School of Basic Medical Sciences, Tianjin Medical University, 22 Qixiangtai Rd, Heping District, Tianjin 300070, China, Email zhangjian7@tmu.edu.cn

\*D.J., P.B., N.W., J.L. and Y.S., J.Z., Y.Y. contributed equally to this article.

The Data Supplement is available with this article at <https://www.ahajournals.org/doi/suppl/10.1161/CIRCRESAHA.120.316784>.

For Sources of Funding and Disclosures, see page 1334.

© 2020 American Heart Association, Inc.

*Circulation Research* is available at [www.ahajournals.org/journal/res](http://www.ahajournals.org/journal/res)

## Novelty and Significance

### What Is Known?

- Pulmonary vascular remodeling is the main pathological hallmark of pulmonary arterial hypertension contributing to the detrimental effects of this disease.
- Vascular inflammation mediated by perivascular inflammatory cells such as macrophages and T cells is an important driver for pulmonary vascular remodeling.
- Niacin exhibits anti-inflammatory and vasodilating effects by releasing prostaglandin D<sub>2</sub>.

### What New Information Does This Article Contribute?

- Niacin ameliorates experimental pulmonary hypertension in rodents through the suppression of pulmonary artery remodeling.
- Niacin boosts prostaglandin D<sub>2</sub> generation in lung tissue mainly through H-PGDS (hematopoietic PGD<sub>2</sub> synthases) in macrophages.
- Macrophage-specific deletion of H-PGDS abolishes the protective effects of niacin against pulmonary hypertension in mice.

Niacin is a longstanding lipid-lowering drug with a relatively safe pharmacological profile. In this study, we demonstrate that niacin suppresses pulmonary vascular remodeling in rodent models of pulmonary arterial hypertension. Niacin stimulates prostaglandin D<sub>2</sub> release through H-PGDS, but not lipocalin-type PGDS in lung tissues. H-PGDS is expressed dominantly in infiltrating macrophages in the lungs from pulmonary arterial hypertension. Macrophage-specific deletion of H-PGDS aggravates hypoxia-induced pulmonary hypertension in mice and abrogates niacin's protective effect on pulmonary hypertension. These findings support the potentially beneficial effect for niacin therapy on pulmonary arterial hypertension.

### Nonstandard Abbreviations and Acronyms

<b>DP1, DP2</b>	PGD2 receptor subtypes 1 and 2, respectively
<b>H-PGDS</b>	hematopoietic PGD <sub>2</sub> synthases
<b>HySu</b>	hypoxia/SU5416
<b>L-PGDS</b>	lipocalin-type PGD2 synthases
<b>PAH</b>	pulmonary arterial hypertension
<b>PASMCs</b>	pulmonary arterial vascular smooth muscle cells
<b>PGD<sub>2</sub></b>	prostaglandin D <sub>2</sub>
<b>PH</b>	pulmonary hypertension
<b>RV/LV+S</b>	ratio of the weight of the right ventricle to the weight of the left ventricle plus septum
<b>RVSP</b>	right ventricular systolic pressure
<b>WT</b>	wild-type
<b>α-SMA</b>	α-smooth muscle actin

reduction of cardiovascular events by niacin treatment is associated with but also due to effects beyond its lipid-lowering effect.<sup>9,10</sup> Both immediate- and extended-release niacin significantly decrease systolic and diastolic blood pressure in hyperlipidemic hypertensive or normotensive individuals.<sup>11–14</sup> Niacin also causes cutaneous vasodilation (facial flushing), which is mediated by enhanced prostaglandin D<sub>2</sub> (PGD<sub>2</sub>) production.<sup>15,16</sup> PGD<sub>2</sub> is an arachidonic acid metabolite, derived from the sequential reaction of cyclooxygenase and two PGDS (PGD<sub>2</sub> synthases), the H-PGDS (hematopoietic PGDS), and the L-PGDS (lipocalin-type PGDS).<sup>17</sup> Moreover, niacin displays anti-inflammatory properties, including regulation of the expression of cell adhesion molecules and macrophage polarization.<sup>18–21</sup> Blockage of PGD<sub>2</sub> signaling suppresses niacin-induced vasodilation and the resolution of inflammation.<sup>22–25</sup> However, whether or not niacin has a therapeutic effect on PAH remains to be determined.

In this study, we found that niacin ameliorated pulmonary hypertension (PH) induced either by administration of a VEGFR (vascular endothelial growth factor receptor) inhibitor SU5416 and chronic hypoxia or by a toxic alkaloid monocrotaline in rodents, through the suppression of pulmonary artery remodeling. Ablation of H-PGDS, but not L-PGDS, markedly reduced PGD<sub>2</sub> production in lung tissues and abolished the beneficial effects of niacin on SU5416/hypoxia-induced PH in mice. Furthermore, we found that H-PGDS was expressed mainly in infiltrating macrophages in the lung tissues of hypoxia-challenged

by these inflammatory cells, such as cytokines, chemokines, and prostaglandins, may be responsible for the phenotypic transformation of pulmonary artery endothelial cells and PASMCs,<sup>5–7</sup> but the mechanisms underlying this transformation are still not completely understood.

Niacin (nicotinic acid), also known as vitamin B3, is a pharmacological agent for the treatment of various cardiovascular conditions, particularly the hyperlipidemias.<sup>8</sup> Accumulated evidence from clinical trials suggests that

mice and patients with idiopathic PAH. Additionally, specific deletion of H-PGDS in macrophages interrupted the protective effect of niacin on SU5416/hypoxia-induced PH in mice. These findings suggest that niacin alleviates PAH progression through promoting H-PGDS-dependent release of PGD<sub>2</sub> from macrophages.

## METHODS

Detailed Methods are available in the [Data Supplement](#). The data that support the findings of this study are available from the corresponding authors on reasonable request.

## RESULTS

### Niacin Attenuates the Development of Hypoxia/SU5416-Induced PH in Mice

To explore the role of niacin in the development of PH, we examined pulmonary hemodynamic and histological changes in hypoxia/SU5416 (HySu)-exposed mice after niacin treatment (Figure 1A). Niacin dramatically attenuated the PH by reducing right ventricular systolic pressure (RVSP) to 75.7% (niacin,  $28.43 \pm 1.36$  mmHg versus vehicle,  $37.57 \pm 1.07$  mmHg;  $P=0.0017$ ; Figure 1B) and ratio of the weight of the right ventricle to the weight of the left ventricle plus septum (RV/LV+S) to 83.3% (niacin,  $0.35 \pm 0.02$  versus vehicle,  $0.42 \pm 0.15$ ;  $P=0.0070$ ; Figure 1C). Furthermore, niacin treatment led to a modest suppression of HySu-induced pulmonary vascular remodeling in both small pulmonary arteries and arterioles, as indicated by decreased pulmonary vascular wall thickness (media/cross-sectional area; Figure 1D and 1E), muscularization (Figure 1F), and  $\alpha$ -SMA ( $\alpha$ -smooth muscle actin) expression (Figure 1G), but had no significant effect on the deposition of perivascular matrix proteins in lungs, such as collagen-1, fibronectin, and tenascin-C (Figure 1A through 1E in the [Data Supplement](#)). Interestingly, niacin markedly suppressed HySu-induced PASMCs hypertrophy by reducing PASMC size (Figure 1H). Moreover, niacin treatment markedly inhibited HySu-induced PASMC proliferation by reducing BrdU (bromo-deoxyuridine) incorporation rate (Figure 1I and 1J in the [Data Supplement](#)), and suppressed the perivascular inflammatory response in lung tissues in HySu-challenged mice, including macrophage and neutrophil infiltration (Figure 1K through 1L in the [Data Supplement](#)).

### Niacin Suppresses Progression of Monocrotaline- and HySu-Induced PH and PA Remodeling In Rats

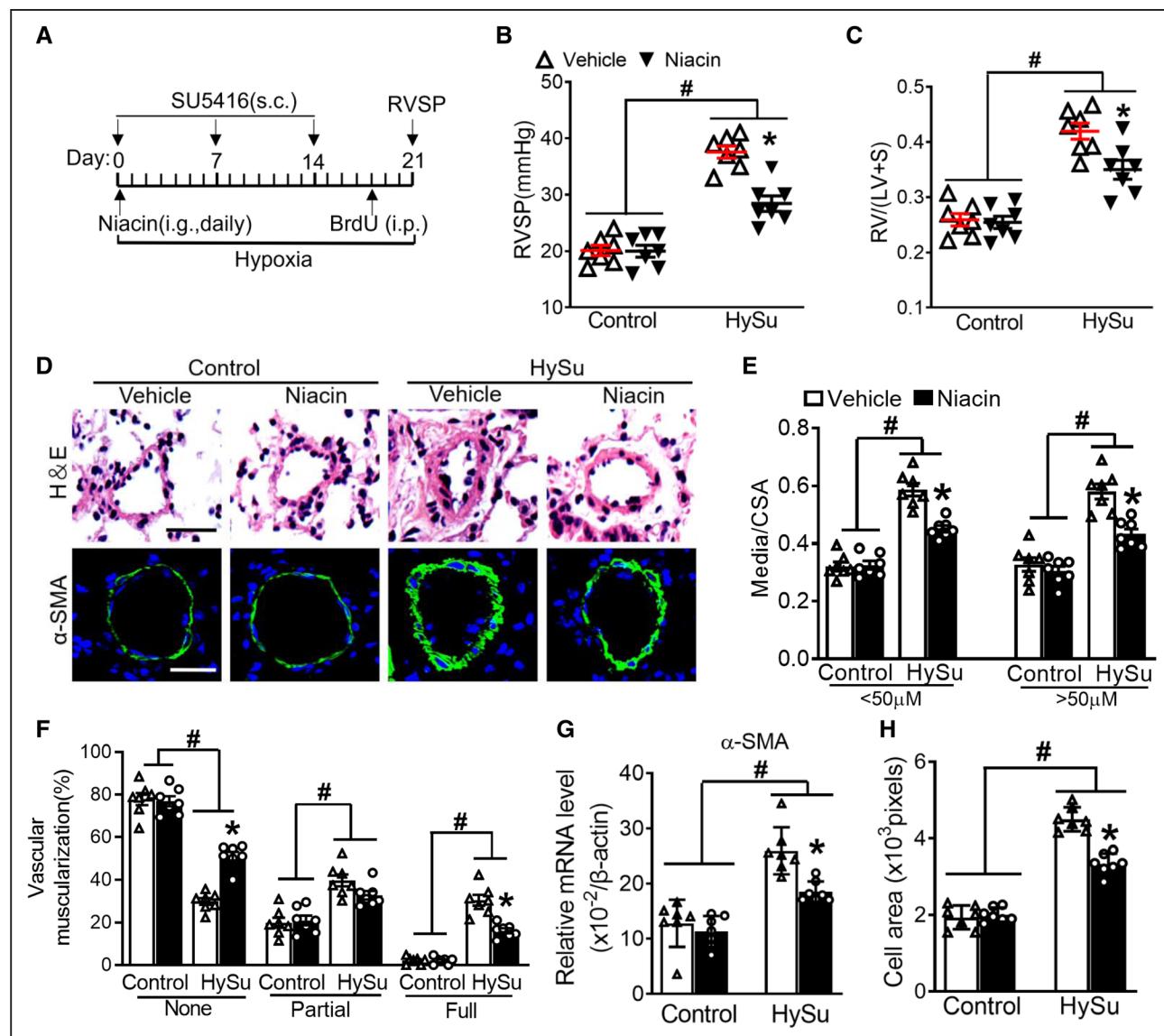
We then explored the therapeutic effect of niacin on PH and pulmonary vascular remodeling in monocrotaline-treated rats. A dose of 600 mg/kg niacin was

administered to the monocrotaline-treated rats once a day at the beginning of the third week after monocrotaline treatment (Figure 2A). As previously reported,<sup>7</sup> monocrotaline challenge significantly elevated pulmonary arterial pressure in rats (Figure 2B and 2C). We found that niacin administration markedly attenuated the increased RVSP (niacin,  $30.20 \pm 0.88$  mmHg versus vehicle,  $35.20 \pm 1.04$  mmHg;  $P=0.0030$ ; Figure 2B), RV/LV+S ratio (niacin,  $0.32 \pm 0.01$  versus vehicle,  $0.40 \pm 0.01$ ;  $P=0.0001$ ; Figure 2C), and pulmonary vascular wall thickness in monocrotaline-treated rats (Figure 2D and 2E). Notably, niacin suppressed both PASMC hypertrophy (Figure 2F) and proliferation (Figure IIIA through IIID in the [Data Supplement](#)), and inhibited vascular infiltration of macrophages (Figure IIIE through IIIG in the [Data Supplement](#)), without affecting matrix protein deposition (Figure IIIH in the [Data Supplement](#)), in monocrotaline-challenged rats. Likewise, niacin treatment conferred protection against HySu-induced PH in rats (Figure 2G through 2L). These results show that niacin treatment alleviates the progression of pulmonary vascular remodeling in established pulmonary hypertension mainly through inhibition of PASMC hypertrophy and proliferation.

Prostacyclin analogs, baraprost, iloprost, and treprostinil, are widely used for the treatment of PH in clinic.<sup>26</sup> To investigate whether niacin exhibits synergistic therapeutic effect with prostacyclin analog on PH, niacin and treprostinil were coadministered to monocrotaline-treated rats (Figure IVA in the [Data Supplement](#)). Compared with treprostinil alone, niacin plus treprostinil markedly attenuated the RVSP (niacin and treprostinil,  $24.50 \pm 0.48$  mmHg versus treprostinil alone,  $28.28 \pm 0.86$  mmHg;  $P=0.0025$ ; Figure IVB in the [Data Supplement](#)), RV/LV+S ratio (niacin and treprostinil,  $0.32 \pm 0.01$  versus treprostinil alone,  $0.28 \pm 0.01$ ;  $P=0.0152$ ; Figure IVC in the [Data Supplement](#)), and reduced pulmonary vascular wall thickness (Figure IVD and IVE in the [Data Supplement](#)) and PASMC hypertrophy in monocrotaline-challenged rats (Figure IVF in the [Data Supplement](#)).

### Niacin Ameliorates HySu-Induced PH in Mice Through H-PGDS-Derived PGD<sub>2</sub>

To explore whether niacin protects against PAH through PGD<sub>2</sub> release, we examined PG production in the lung tissues of the HySu-induced PH mouse model with niacin treatment. Strikingly, niacin significantly enhanced PGD<sub>2</sub> and PGE<sub>2</sub> production in the lungs under normoxic conditions but especially promoted PGD<sub>2</sub> production in HySu-treated mice (Figure 3A, Figure VA in the [Data Supplement](#)). Other PGs, such as PGF<sub>2 $\alpha$</sub> , PGI<sub>2</sub>, and TxA<sub>2</sub> (Thromboxane A2) were not significantly changed by niacin treatment under either normoxic or HySu-exposed conditions (Figure VB through VD in the [Data Supplement](#)).

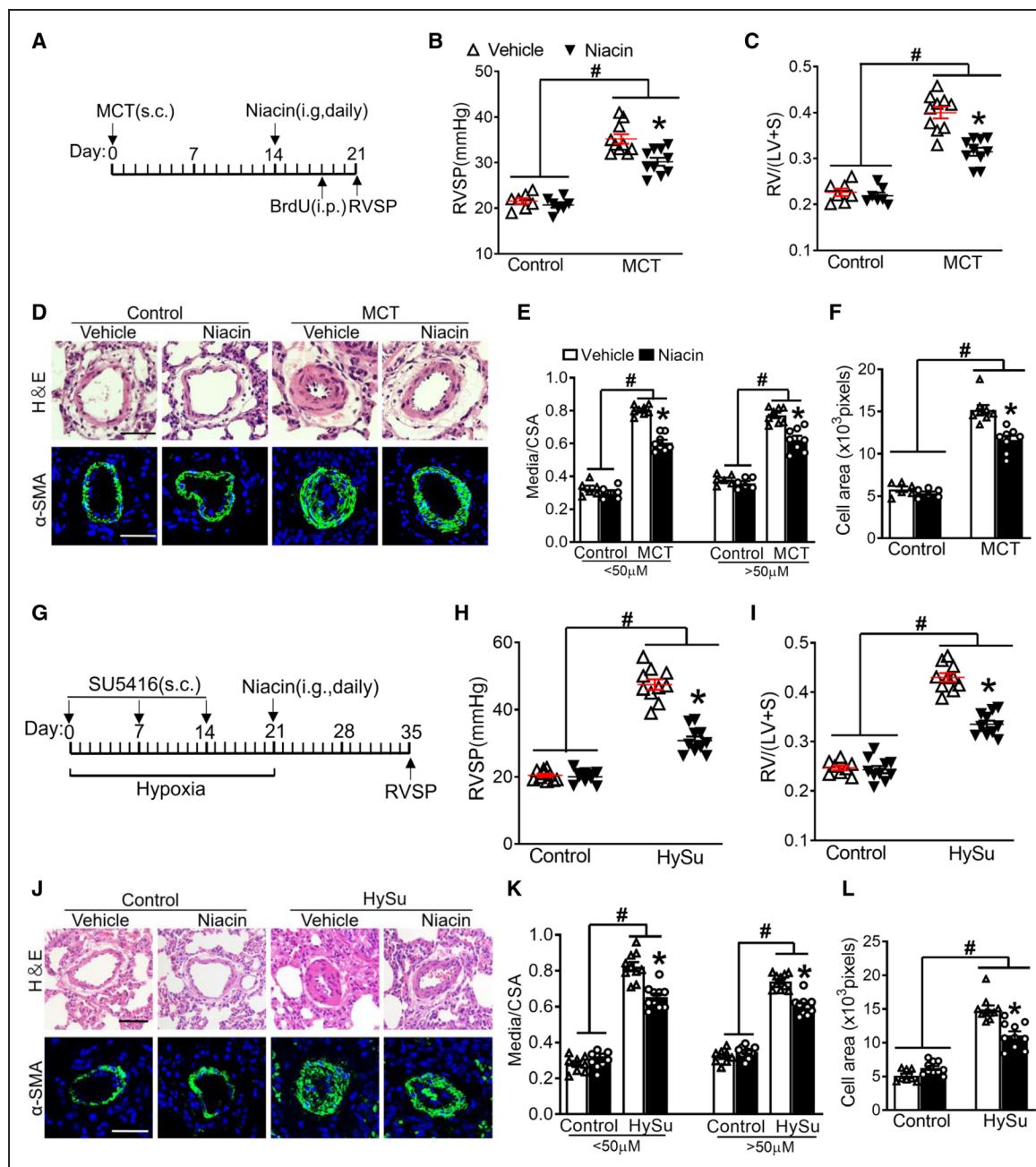


**Figure 1. Niacin attenuates the development of hypoxia/SU5416 (HySu)-induced pulmonary hypertension in mice.**

**A**, Protocol for administration of niacin to mice exposed to HySu. **B**, Right ventricular systolic pressure (RVSP) in mice exposed to HySu with niacin treatment. \* $P<0.05$  vs vehicle; # $P<0.05$  vs control ( $n=7$  each). **C**, Ratio of the weight of the right ventricle to the weight of the left ventricle plus septum (RV/LV+S) in mice exposed to HySu with niacin treatment. \* $P<0.05$  vs vehicle; # $P<0.05$  vs control ( $n=7$  each). **D**, Representative hematoxylin and eosin (H&E)-stained sections and immunofluorescence images of  $\alpha$ -SMA ( $\alpha$ -smooth muscle actin; green) expression from mice exposed to HySu with niacin treatment. Scale bars: 25  $\mu$ m. **E**, Quantification of the ratio of vascular medial thickness to total vessel size for mice exposed to HySu with niacin treatment. \* $P<0.05$  vs vehicle; # $P<0.05$  vs control ( $n=7$  each). **F**, Proportion of none, partially, and fully muscularized pulmonary arteries (PAs; diameter: 20–50  $\mu$ m) in mice exposed to HySu with niacin treatment. \* $P<0.05$  vs vehicle; # $P<0.05$  vs control ( $n=7$  each). **G**,  $\alpha$ -SMA mRNA levels in the lungs of mice exposed to HySu with niacin treatment. \* $P<0.05$  vs vehicle; # $P<0.05$  vs control ( $n=7$  each). **H**, Quantification of pulmonary arterial vascular smooth muscle cell size in PAs from mice exposed to HySu with niacin treatment. \* $P<0.05$  vs vehicle; # $P<0.05$  vs control ( $n=7$  each). Data represent mean  $\pm$  SEM. Statistical significance was evaluated by Kruskal-Wallis tests followed by Dunn test. The exact  $P$  values are listed in Statistic Dataset in the [Data Supplement](#). BrdU indicates bromo-deoxyuridine; and CSA, cross-sectional area.

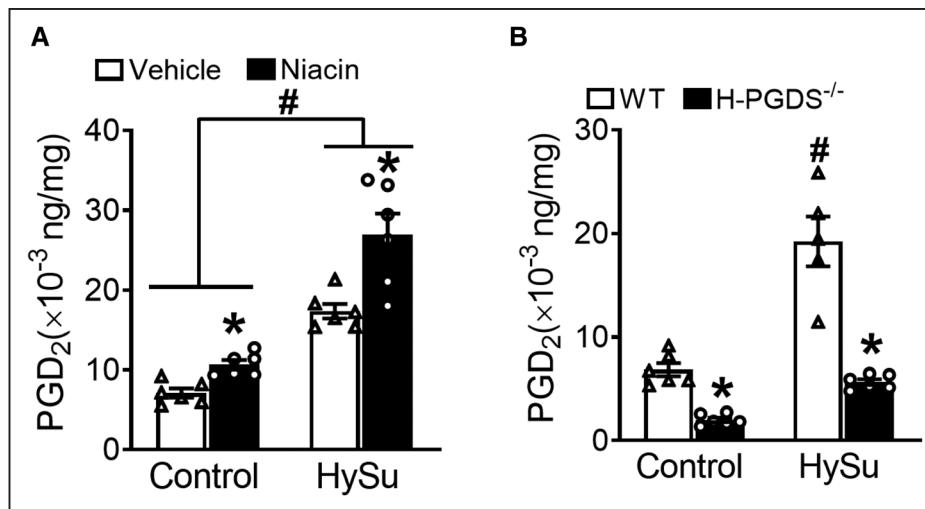
Two distinct PGDSs, H-PGDS and L-PGDS, mediate the biosynthesis of PGD<sub>2</sub>.<sup>17</sup> PGD<sub>2</sub> production was markedly reduced in lung tissue from H-PGDS<sup>-/-</sup> mice (Figure 3B), but not in L-PGDS<sup>-/-</sup> mice (data not shown), at both normoxic and HySu-exposed conditions. Strikingly, upon HySu exposure (Figure 4A), H-PGDS<sup>-/-</sup> mice also developed more severe PH, with significant elevations in RVSP (H-PGDS<sup>-/-</sup>,  $45.00 \pm 1.63$  mm Hg versus wild-type

(WT),  $35.43 \pm 1.23$  mm Hg;  $P=0.0017$ ; Figure 4B), and RV/LV+S (H-PGDS<sup>-/-</sup>,  $0.49 \pm 0.02$  versus WT,  $0.42 \pm 0.01$ ;  $P=0.0111$ ; Figure 4C). Moreover, H-PGDS deletion promoted HySu-induced pulmonary vascular remodeling in mice, as evidenced by increased pulmonary vascular wall thickness (Figure 4D and 4E), muscularization (Figure 4F), enlarged PASMCs (Figure 4G), and enhanced vascular macrophage infiltration (Figure VIA through



**Figure 2. Niacin suppresses progression of monocrotaline (MCT)- and hypoxia/SU5416 (HySu)-induced pulmonary hypertension and PA remodeling in rats.**

**A**, Protocol for administration of niacin to MCT-challenged rats. **B** and **C**, Effect of niacin administration on right ventricular systolic pressure (RVSP) and ratio of the weight of the right ventricle to the weight of the left ventricle plus septum (RV/LV+S) ratio in monocrotaline -treated rats. \*P<0.05 vs vehicle; #P<0.05 vs control (control+vehicle: n=7, control+niacin: n=7, MCT+vehicle: n=10, MCT+niacin: n=10). **D**, Representative hematoxylin (H&E)-stained lung sections and immunofluorescence images of α-SMA (α-smooth muscle actin; green) expression of MCT-injected rats treated with niacin. Scale bars: 25 μm. **E**, Quantification of the ratio of vascular medial thickness to total vessel size in MCT-challenged rats after niacin treatment. \*P<0.05 vs vehicle; #P<0.05 vs control (control+vehicle: n=6, control+niacin: n=6, MCT+vehicle: n=8, MCT+niacin: n=8). **F**, Quantification of pulmonary arterial vascular smooth muscle cell (PASMC) size in PAs of MCT-challenged rats after niacin treatment. \*P<0.05 vs vehicle; #P<0.05 vs control (control+vehicle: n=6, control+niacin: n=6, MCT+vehicle: n=8, MCT+niacin: n=8). **G**, Protocol for administration of niacin to HySu-challenged rats. **H** and **I**, Effect of niacin treatment on RVSP and RV/LV+S ratio in HySu-challenged rats. \*P<0.05 vs vehicle; #P<0.05 vs control (control+vehicle: n=8, control+niacin: n=10, HySu+vehicle: n=10, HySu+niacin: n=10). **J**, Representative H&E-stained lung sections and immunofluorescence images of α-SMA (green) expression of HySu-challenged rats after niacin treatment. Scale bars: 25 μm. **K**, Quantification of the ratio of vascular medial thickness to total vessel size in HySu-exposed rats after niacin treatment. \*P<0.05 vs vehicle; #P<0.05 vs control (control+vehicle: n=8, control+niacin: n=10, HySu+vehicle: n=10, HySu+niacin: n=10). **L**, Quantification of PASMC size in PAs of HySu-exposed rats after niacin treatment. \*P<0.05 vs vehicle; #P<0.05 vs control (control+vehicle: n=8, control+niacin: n=10, HySu+vehicle: n=10, HySu+niacin: n=10). Data represent mean±SEM. Statistical significance was evaluated by Kruskal-Wallis tests followed by Dunn test. The exact P values are listed in Statistic Dataset in the [Data Supplement](#). BrdU indicates bromo-deoxyuridine; and CSA, cross-sectional area.



**Figure 3. Niacin promotes prostaglandin D<sub>2</sub> (PGD<sub>2</sub>) generation in SU5416/hypoxia-induced pulmonary arterial hypertension through H-PGDS (hematopoietic PGD<sub>2</sub> synthases) in mice.**

**A**, PGD<sub>2</sub> production in lung tissues from mice exposed to hypoxia/SU5416 (HySu) with niacin treatment. \*P<0.05 vs vehicle; #P<0.05 vs control (n=6 each). **B**, PGD<sub>2</sub> production in lung tissues from wild-type (WT) and H-PGDS<sup>-/-</sup> mice exposed to HySu. \*P<0.05 vs WT; #P<0.05 vs control (control+WT: n=6, control+H-PGDS<sup>-/-</sup>: n=6, HySu+WT: n=5, HySu+H-PGDS<sup>-/-</sup>: n=6). Data represent mean±SEM. Statistical significance was evaluated by Kruskal-Wallis tests followed by Dunn test. The exact P values are listed in Statistic Dataset in the Data Supplement.

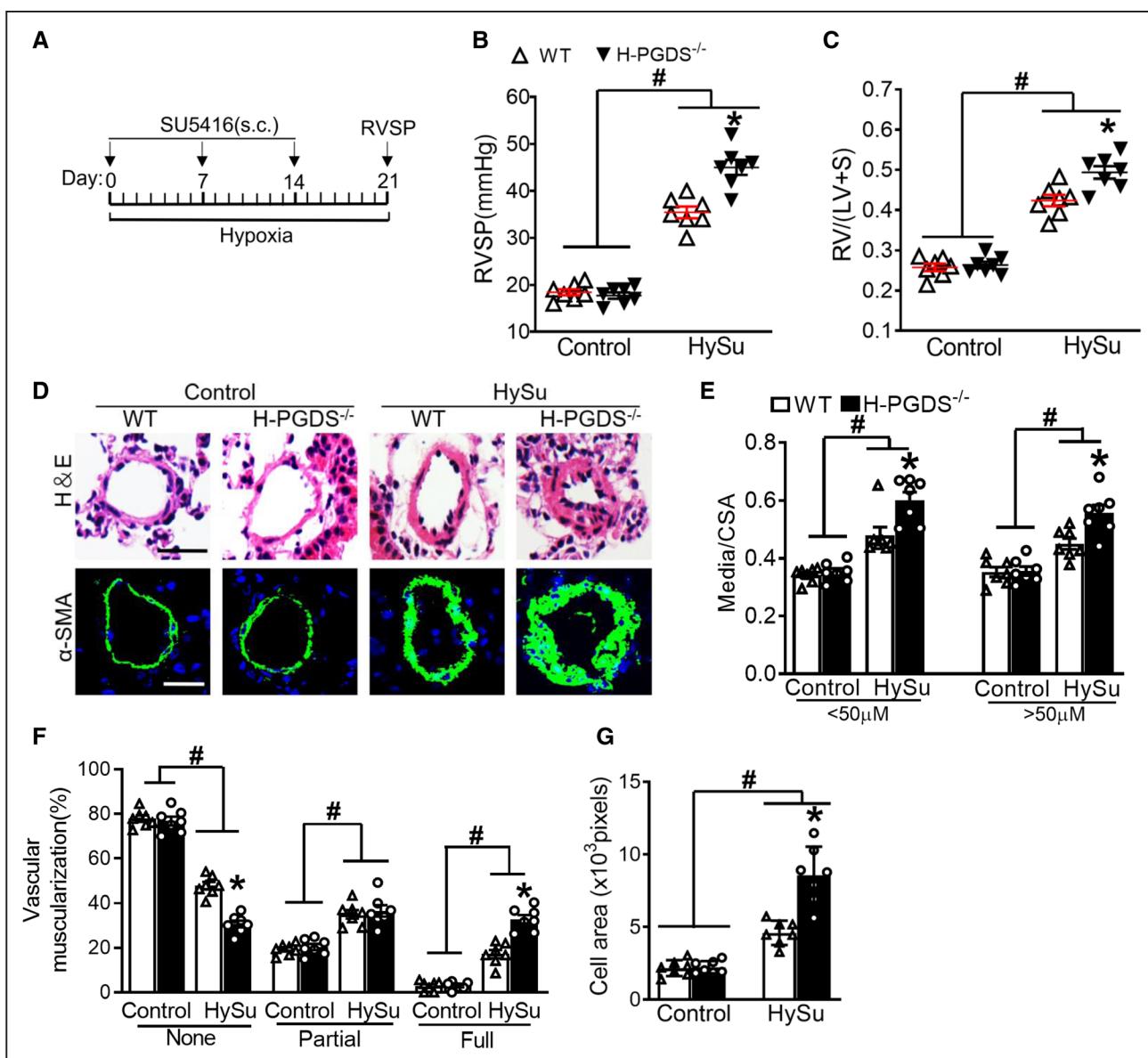
VIC in the Data Supplement). In contrast, L-PGDS deficiency had no significant impact on RVSP, RV/LV+S, and vascular wall thickness in HySu-exposed mice (data not shown). We then explored whether niacin protected against PAH through boosting H-PGDS-derived PGD<sub>2</sub>. As shown in Figure 5, niacin lost its beneficial effects on PH development in H-PGDS<sup>-/-</sup> mice when exposed to HySu conditions (Figure 5A through 5E). Indeed, H-PGDS deficiency abolished niacin-induced PGD<sub>2</sub> production in the lungs of HySu-treated mice (Figure 5F). Thus, these results indicate that niacin alleviates the progression of PAH through H-PGDS-dependent PGD<sub>2</sub> release.

### H-PGDS in Macrophages Mediates Niacin's Protective Effect on HySu-Induced PH in Mice

H-PGDS is expressed in some hematopoietic lineage cells, such as mast cells,<sup>27</sup> macrophages,<sup>21,28</sup> and megakaryoblastic cells.<sup>29,30</sup> In HySu-exposed mice and patients with idiopathic PAH, massive macrophages, mast cells, and neutrophils had infiltrated into both the perivascular area of lung tissues and areas surrounding pulmonary alveoli (Figure 6). We observed H-PGDS staining was nicely co-localized with CD68<sup>+</sup> macrophages and some c-kit<sup>+</sup> or Chymase<sup>+</sup> mast cells, but not with Ly6G<sup>+</sup> neutrophils, in lung tissues from HySu-treated mice (Figure 6A through 6F) and patients with idiopathic PAH (Figure 6G through 6L).

Macrophage-specific deletion of H-PGDS (H-PGDS<sup>F/F</sup>LysM<sup>Cre</sup>) significantly reduced pulmonary PGD<sub>2</sub> production by at least 50% (Figure 7A), whereas H-PGDS ablation in mast cells (H-PGDS<sup>F/F</sup>Mcpt5<sup>Cre</sup> [mast cell protease 5]) had no significant influence on PGD<sub>2</sub> levels in mouse lung tissues under both normoxic and HySu-exposed

conditions (data not shown). This effect might be due to a lack of Mcpt5-cre transgene expression in mast cells of the mucosal (intraepithelial) type.<sup>31</sup> Kit<sup>W-sh/W-sh</sup> mice lack mature mast cells because of an inversion mutation in the Kit gene promoter.<sup>32</sup> Indeed, H-PGDS was dominantly expressed in macrophages in the lung tissues of Kit<sup>W-sh/W-sh</sup> mice. Surprisingly, global deletion of mast cells alleviated HySu-induced PH in Kit<sup>W-sh/W-sh</sup> mice via a significant reduction in RVSP, RV/LV+S, and pulmonary vascular wall thickness, but had no markedly influence on PGD<sub>2</sub> production in lung tissues (data not shown), suggesting PGD<sub>2</sub> is dominantly generated from infiltrated macrophages in lungs upon hypoxia exposure. As expected, when compared with controls, H-PGDS<sup>F/F</sup>LysM<sup>Cre</sup> mice developed more severe PH when exposed to HySu, with significant elevations in RVSP (H-PGDS<sup>F/F</sup>LysM<sup>Cre</sup>, 44.29±2.42 mm Hg versus H-PGDS<sup>F/F</sup>, 36.86±1.32 mm Hg; P=0.0420; Figure 7B) and RV/LV+S (H-PGDS<sup>F/F</sup>LysM<sup>Cre</sup>, 0.48±0.01 versus H-PGDS<sup>F/F</sup>, 0.40±0.01; P=0.0023; Figure 7C), and increased pulmonary vascular wall thickness (Figure 7D and 7E) and PASMC hypertrophy (Figure 7F). We also observed that niacin treatment failed to attenuate increased RVSP (Figure 8A), RV/LV+S (Figure 8B), pulmonary vascular wall thickness, and PASMC hypertrophy (Figure 8C through 8E) in H-PGDS<sup>F/F</sup>LysM<sup>Cre</sup> mice exposed to HySu. Indeed, macrophage-specific deletion of H-PGDS also abolished niacin-induced PGD<sub>2</sub> production in the lungs of HySu-treated mice (Figure 8F). Furthermore, we explored whether H-PGDS in bone marrow-derived macrophages mediated protective effect of niacin in experimental PH using bone marrow transplantation approach (Figure VIIA in the Data Supplement). Indeed, transplantation of H-PGDS<sup>-/-</sup> bone marrow to WT (KO [knockout]→WT) exacerbated HySu-induced

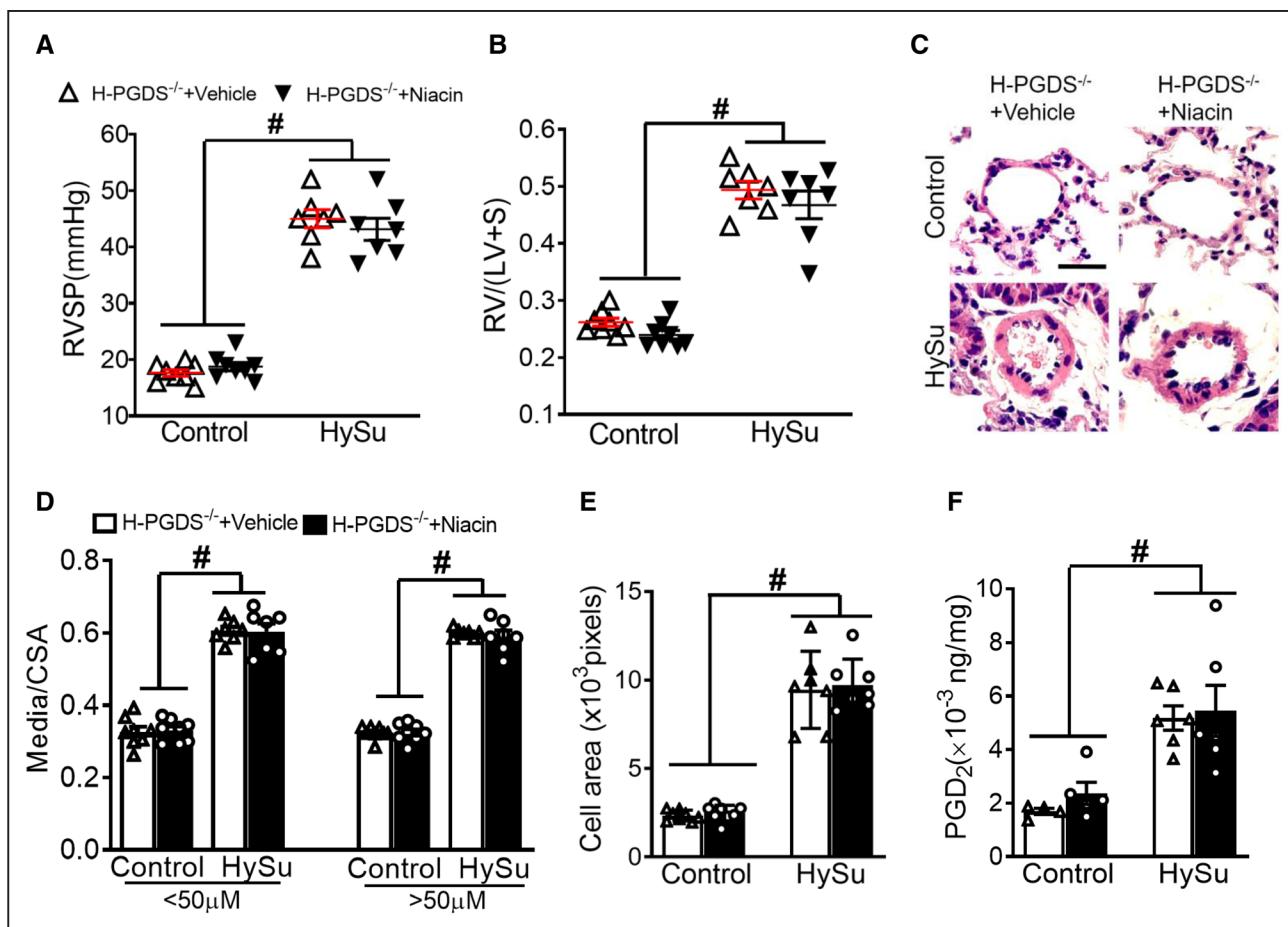


**Figure 4. H-PGDS (hematopoietic prostaglandin D<sub>2</sub> synthases) deficiency aggravates hypoxia/SU5416 (HySu)–induced pulmonary hypertension (PH) and PA remodeling in mice.**

**A**, Protocol for HySu-induced PH in mice. **B**, Right ventricular systolic pressure (RVSP) in H-PGDS<sup>-/-</sup> and wild-type (WT) mice after exposure to HySu. \*P<0.05 vs WT; #P<0.05 vs control (n=7 each). **C**, Ratio of the weight of the right ventricle to the weight of the left ventricle plus septum (RV/LV+S) in H-PGDS<sup>-/-</sup> and WT mice after exposure to HySu. \*P<0.05 vs WT; #P<0.05 vs control (n=7 each). **D**, Representative hematoxylin and eosin (H&E)-stained sections and immunofluorescence images of α-SMA (α-smooth muscle actin; green) expression from H-PGDS<sup>-/-</sup> and WT mice exposed to HySu. Scale bars: 25 μm. **E**, Quantification of the ratio of vascular medial thickness to total vessel size for the HySu exposure model. \*P<0.05 vs WT; #P<0.05 vs control (n=7 each). **F**, Proportion of none, partially, and fully muscularized PAs (diameter: 20–50 μm) in mice exposed to HySu. \*P<0.05 vs WT; #P<0.05 vs control (n=7 each). **G**, Quantification of pulmonary arterial vascular smooth muscle cell size in PAs from mice exposed to HySu. \*P<0.05 vs WT; #P<0.05 vs control (n=7 each). Data represent mean±SEM. Statistical significance was evaluated by Kruskal-Wallis tests followed by Dunn test. The exact P values are listed in Statistic Dataset in the [Data Supplement](#). CSA indicates cross-sectional area.

PH in mice as compared to WT→WT transplantation, as evidenced by significant elevated RVSP (Figure VIIB in the [Data Supplement](#)) and RV/LV+S (Figure VIIIC in the [Data Supplement](#)), and increased pulmonary vascular wall thickness (Figure VIID and VIIIE in the [Data Supplement](#)) and PASMC hypertrophy (Figure VIIIF in the [Data Supplement](#)). However, niacin had no notable protective effects on HySu-challenged KO→WT mice (Figure VIIB through

VIIIF in the [Data Supplement](#)). Consistently, coculture with niacin-treated macrophages dramatically attenuated hypoxia-induced PASMC hypertrophy compared with untreated macrophages, as shown by decreased cell size (Figure VIIIA and VIIIB in the [Data Supplement](#)) and protein/DNA ratio (Figure VIIIC in the [Data Supplement](#)), and downregulated α-SMA and SM22 (smooth muscle 22) protein levels (Figure VIID through VIIIF in the [Data](#)



**Figure 5. Effect of niacin on hypoxia/SU5416 (HySu)-induced pulmonary hypertension and PA remodeling in H-PGDS (hematopoietic prostaglandin D<sub>2</sub> synthases)<sup>-/-</sup> mice.**

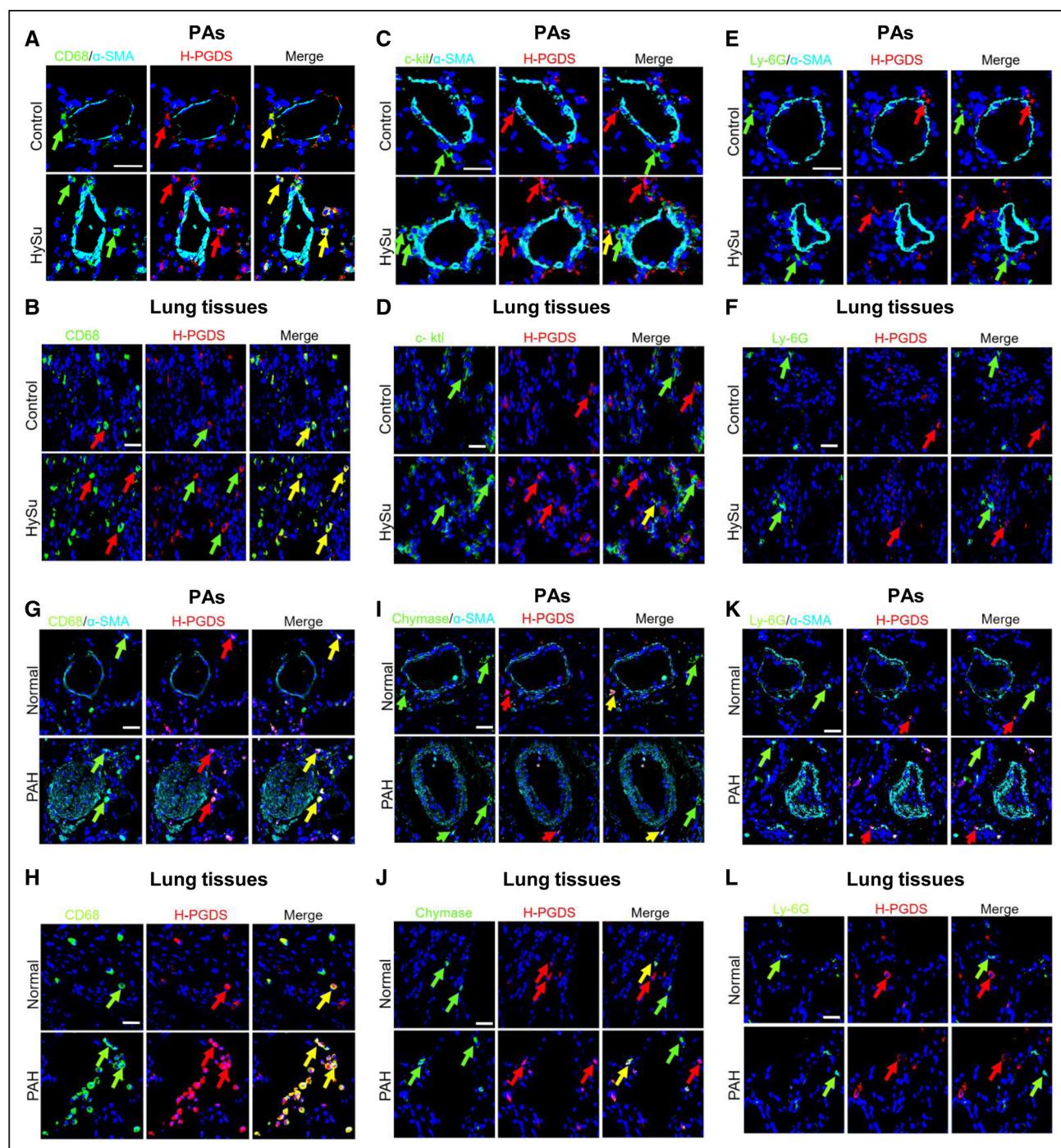
**A**, Right ventricular systolic pressure (RVSP) in H-PGDS<sup>-/-</sup> and wild-type (WT) mice exposed to HySu with niacin treatment. #P<0.05 vs control (control+H-PGDS<sup>-/-</sup>+vehicle: n=8, control+H-PGDS<sup>-/-</sup>+niacin: n=8, HySu+H-PGDS<sup>-/-</sup>+vehicle: n=7, HySu+H-PGDS<sup>-/-</sup>+niacin: n=7). **B**, Ratio of the weight of the right ventricle to the weight of the left ventricle plus septum (RV/LV+S) in H-PGDS<sup>-/-</sup> and WT mice exposed to HySu with niacin treatment. #P<0.05 vs control (control+H-PGDS<sup>-/-</sup>+vehicle: n=8, control+H-PGDS<sup>-/-</sup>+niacin: n=8, HySu+H-PGDS<sup>-/-</sup>+vehicle: n=7, HySu+H-PGDS<sup>-/-</sup>+niacin: n=7). **C**, Representative hematoxylin and eosin (H&E)-stained sections from H-PGDS<sup>-/-</sup> and WT mice exposed to HySu with niacin treatment. Scale bars: 25 μm. **D**, Quantification of the ratio of vascular medial thickness to total vessel size for the HySu exposure model. #P<0.05 vs control (control+H-PGDS<sup>-/-</sup>+vehicle: n=8, control+H-PGDS<sup>-/-</sup>+niacin: n=8, HySu+H-PGDS<sup>-/-</sup>+vehicle: n=7, HySu+H-PGDS<sup>-/-</sup>+niacin: n=7). **E**, Quantification of pulmonary arterial vascular smooth muscle cell size in PAs for the HySu exposure model. #P<0.05 vs control (control+H-PGDS<sup>-/-</sup>+vehicle: n=8, control+H-PGDS<sup>-/-</sup>+niacin: n=8, HySu+H-PGDS<sup>-/-</sup>+vehicle: n=7, HySu+H-PGDS<sup>-/-</sup>+niacin: n=7). **F**, Effect of niacin on prostaglandin D<sub>2</sub> generation in the lungs of H-PGDS<sup>-/-</sup> and WT mice exposed to HySu. #P<0.05 vs control (control+H-PGDS<sup>-/-</sup>+vehicle: n=4, control+H-PGDS<sup>-/-</sup>+niacin: n=5, HySu+H-PGDS<sup>-/-</sup>+vehicle: n=6, HySu+H-PGDS<sup>-/-</sup>+niacin: n=6). Data represent mean±SEM. Statistical significance was evaluated by Kruskal-Wallis tests followed by Dunn test. The exact P values are listed in Statistic Dataset in the [Data Supplement](#). CSA indicates cross-sectional area.

Supplement). Taken together, these results demonstrate that niacin alleviates HySu-induced PH in mice through H-PGDS-dependent PGD<sub>2</sub> release in macrophages.

### Niacin Induces PGD<sub>2</sub> Release in Macrophages Through GPR109A Activation

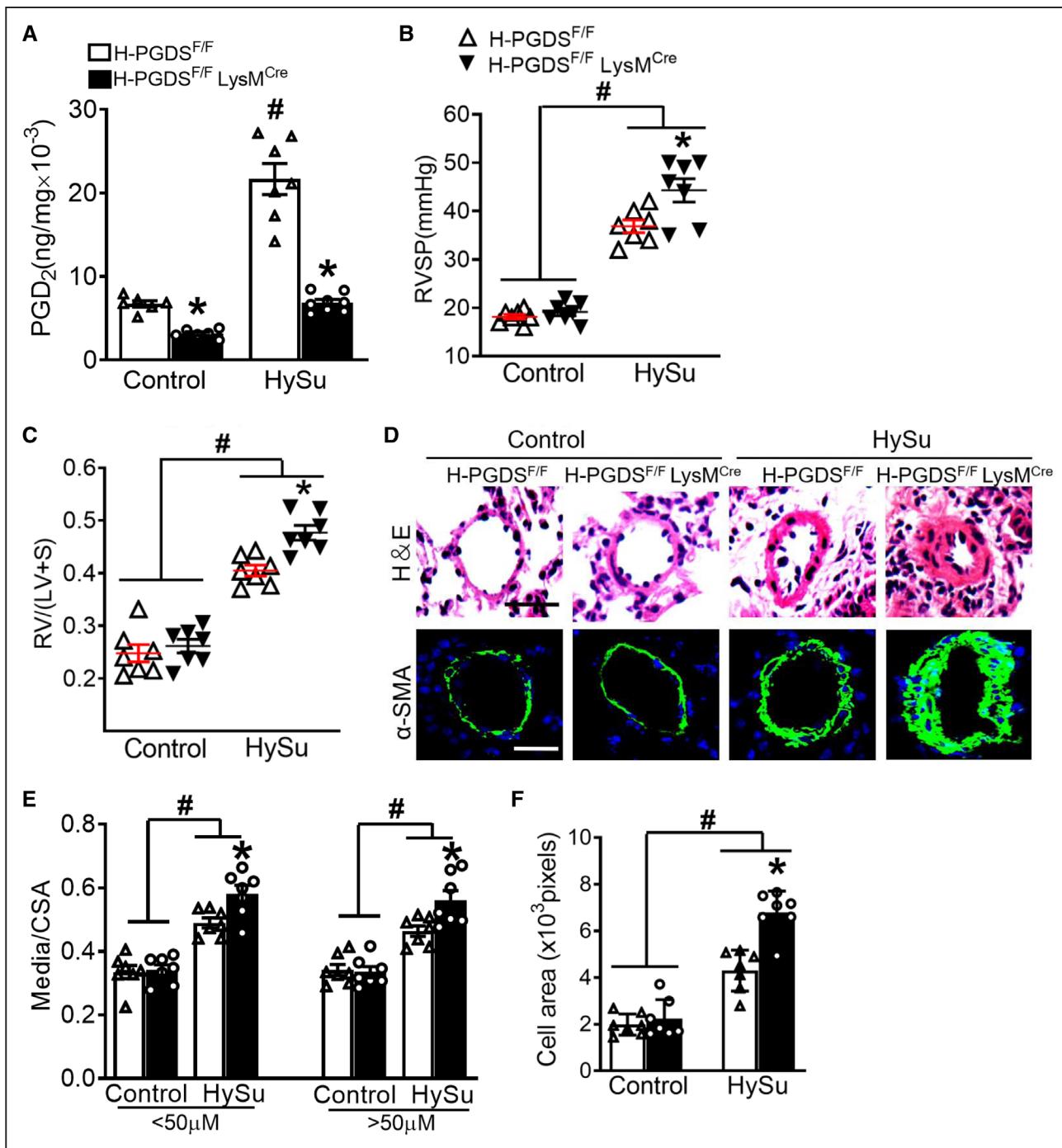
β-arrestin 1 facilitates niacin-induced flushing in mice through G protein-coupled receptor 109A (GPR109A)-mediated PGD<sub>2</sub> release.<sup>33,34</sup> Indeed, niacin receptor GPR109A and H-PGDS were highly expressed in mouse primary macrophages, barely in mouse lung endothelial cells, pulmonary arterial vascular smooth muscle cells,

and lung fibroblasts (Figure IXA and IXB in the [Data Supplement](#)), whereas L-PGDS was detected, but relatively low, in macrophages, mouse pulmonary arterial vascular smooth muscle cells, and mouse lung fibroblasts (Figure IXC in the [Data Supplement](#)). Niacin treatment enhanced PGD<sub>2</sub> production in mouse macrophages, not in other cells tested (Figure IXD in the [Data Supplement](#)). Knockdown of GPR109A (Figure IXE in the [Data Supplement](#)) inhibited niacin-induced β-arrestin 1 membrane recruitment (Figure IXF in the [Data Supplement](#)), abolished its downstream Erk (extracellular signal-regulated kinases)/cPLA<sub>2</sub> (cytosolic phospholipase A2) activity in mouse macrophages (Figure IXG and IXI in



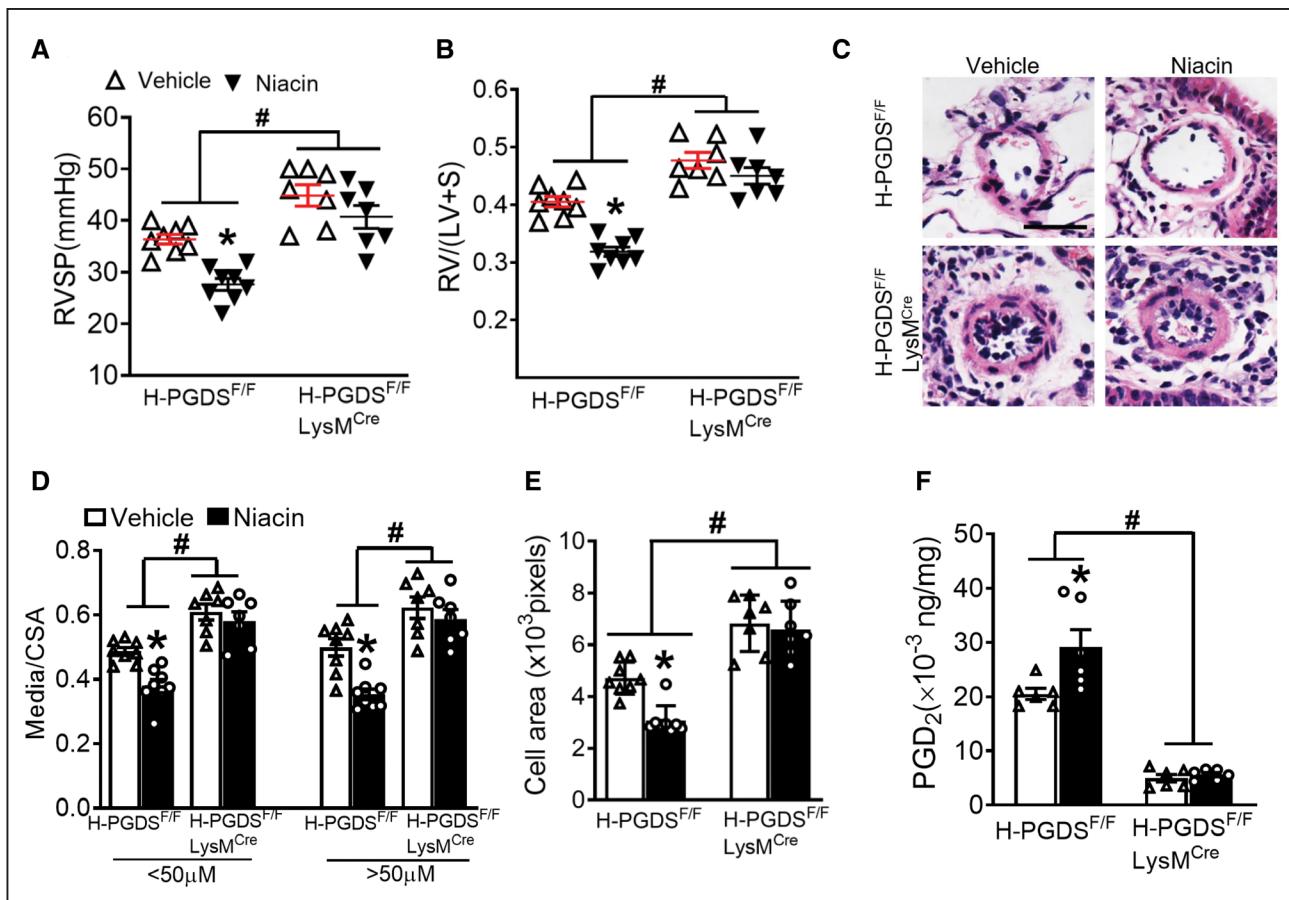
**Figure 6. H-PGDS (hematopoietic prostaglandin D<sub>2</sub> synthases) expression in macrophages, mast cells, and neutrophils in pulmonary arteries (PAs) and lung tissues in hypoxia/SU5416 (HySu)-exposed mice and idiopathic PA hypertension (PAH) patients.**

**A** and **B**, Immunofluorescence detection of CD68 (green) and H-PGDS (red) expression in PAs and lung tissues from HySu-exposed mice. Green arrows: CD68<sup>+</sup> cells. Red arrows: H-PGDS<sup>+</sup> cells. Yellow arrows: CD68<sup>+</sup>/H-PGDS<sup>+</sup> cells. **C** and **D**, Immunofluorescence detection of c-kit (green) and H-PGDS (red) expression in PAs and lung tissues from HySu-exposed mice. Green arrows: c-kit<sup>+</sup> cells. Red arrows: H-PGDS<sup>+</sup> cells. Yellow arrows, c-kit<sup>+</sup>/H-PGDS<sup>+</sup> cells. **E** and **F**, Immunofluorescence detection of Ly-6G (green) and H-PGDS (red) expression in PAs and lung tissues from HySu-exposed mice. Green arrows: Ly-6G<sup>+</sup> cells. Red arrows: H-PGDS<sup>+</sup> cells. Yellow arrows: Ly-6G<sup>+</sup>/H-PGDS<sup>+</sup> cells. **G** and **H**, Immunofluorescence detection of CD68 (green) and H-PGDS (red) expression in PAs and lung tissues from patients with idiopathic PAH. Green arrows: CD68<sup>+</sup> cells. Red arrows: H-PGDS<sup>+</sup> cells. Yellow arrows: CD68<sup>+</sup>/H-PGDS<sup>+</sup> cells. **I** and **J**, Immunofluorescence detection of Chymase (green) and H-PGDS (red) expression in PAs and lung tissues from patients with idiopathic PAH. Green arrows: Chymase<sup>+</sup> cells. Red arrows: H-PGDS<sup>+</sup> cells. Yellow arrows: Chymase<sup>+</sup>/H-PGDS<sup>+</sup> cells. **K** and **L**, Immunofluorescence detection of Ly-6G (green) and H-PGDS (red) expression in PAs and lung tissues from patients with idiopathic PAH. Green arrows: Ly-6G<sup>+</sup> cells. Red arrows: H-PGDS<sup>+</sup> cells. Scale bars: 25  $\mu$ m.



**Figure 7. Macrophage-specific deletion of H-PGDS (hematopoietic prostaglandin D<sub>2</sub> [PGD<sub>2</sub>] synthases) aggravates hypoxia/SU5416 (HySu)-induced pulmonary hypertension and PA remodeling in mice.**

**A**, PGD<sub>2</sub> generation in the lungs of H-PGDS<sup>FF</sup>LysM<sup>Cre</sup> and H-PGDS<sup>FF</sup> mice exposed to HySu. \*P<0.05 vs H-PGDS<sup>FF</sup>; #P<0.05 vs control (control+H-PGDS<sup>FF</sup>: n=6, control+H-PGDS<sup>FF</sup>LysM<sup>Cre</sup>: n=6, HySu+H-PGDS<sup>FF</sup>: n=7, HySu+H-PGDS<sup>FF</sup>LysM<sup>Cre</sup>: n=8). **B**, Right ventricular systolic pressure in H-PGDS<sup>FF</sup>LysM<sup>Cre</sup> and H-PGDS<sup>FF</sup> mice after exposure to HySu. \*P<0.05 vs H-PGDS<sup>FF</sup>; #P<0.05 vs control (n=7 each). **C**, Ratio of the weight of the right ventricle to the weight of the left ventricle plus septum (RV/LV+S) in H-PGDS<sup>FF</sup>LysM<sup>Cre</sup> and H-PGDS<sup>FF</sup> mice after exposure to HySu. \*P<0.05 vs H-PGDS<sup>FF</sup>; #P<0.05 vs control (n=7 each). **D**, Representative hematoxylin and eosin (H&E)-stained sections and immunofluorescence images of α-SMA (α-smooth muscle actin; green) expression from H-PGDS<sup>FF</sup>LysM<sup>Cre</sup> and H-PGDS<sup>FF</sup> mice exposed to HySu. Scale bars: 25 μm. **E**, Quantification of the ratio of vascular medial thickness to total vessel size for the HySu exposure model. \*P<0.05 vs H-PGDS<sup>FF</sup>; #P<0.05 vs control (n=7 each). **F**, Quantification of pulmonary arterial vascular smooth muscle cell size in PAs for the HySu exposure model. \*P<0.05 vs H-PGDS<sup>FF</sup>; #P<0.05 vs control (n=7 each). Data represent mean±SEM. Statistical significance was evaluated by Kruskal-Wallis tests followed by Dunn test. The exact P values are listed in Statistic Dataset in the [Data Supplement](#). CSA indicates cross-sectional area.



**Figure 8. Effect of niacin on hypoxia/SU5416 (HySu)-induced pulmonary hypertension and PA remodeling in H-PGDS (hematopoietic prostaglandin D<sub>2</sub> synthases)<sup>F/F</sup> LysM<sup>Cre</sup> mice.**

**A**, right ventricular systolic pressure (RVSP) in H-PGDS<sup>F/F</sup> LysM<sup>Cre</sup> and H-PGDS<sup>F/F</sup> mice exposed to HySu with niacin treatment. \*P<0.05 vs vehicle; #P<0.05 vs H-PGDS<sup>F/F</sup> (H-PGDS<sup>F/F</sup>+vehicle: n=8, H-PGDS<sup>F/F</sup>+niacin: n=8, H-PGDS<sup>F/F</sup>LysM<sup>Cre</sup>+vehicle: n=7, H-PGDS<sup>F/F</sup>LysM<sup>Cre</sup>+niacin: n=7). **B**, Ratio of the weight of the right ventricle to the weight of the left ventricle plus septum (RV/LV+S) in H-PGDS<sup>F/F</sup> LysM<sup>Cre</sup> and H-PGDS<sup>F/F</sup> mice exposed to HySu with niacin treatment. \*P<0.05 vs vehicle; #P<0.05 vs H-PGDS<sup>F/F</sup> (H-PGDS<sup>F/F</sup>+vehicle: n=8, H-PGDS<sup>F/F</sup>+niacin: n=8, H-PGDS<sup>F/F</sup>LysM<sup>Cre</sup>+vehicle: n=7, H-PGDS<sup>F/F</sup>LysM<sup>Cre</sup>+niacin: n=7). **C**, Representative hematoxylin and eosin-stained sections from H-PGDS<sup>F/F</sup>LysM<sup>Cre</sup> and H-PGDS<sup>F/F</sup> mice exposed to HySu with niacin treatment. Scale bar: 25 μm. **D**, Quantification of the ratio of vascular medial thickness to total vessel size for the HySu exposure model. \*P<0.05 vs vehicle; #P<0.05 vs H-PGDS<sup>F/F</sup> (H-PGDS<sup>F/F</sup>+vehicle: n=8, H-PGDS<sup>F/F</sup>+niacin: n=8, H-PGDS<sup>F/F</sup>LysM<sup>Cre</sup>+vehicle: n=7, H-PGDS<sup>F/F</sup>LysM<sup>Cre</sup>+niacin: n=7). **E**, Quantification of pulmonary arterial vascular smooth muscle cell size in PAs for the HySu exposure model. \*P<0.05 vs vehicle; #P<0.05 vs H-PGDS<sup>F/F</sup> (H-PGDS<sup>F/F</sup>+vehicle: n=8, H-PGDS<sup>F/F</sup>+niacin: n=8, H-PGDS<sup>F/F</sup>LysM<sup>Cre</sup>+vehicle: n=7, H-PGDS<sup>F/F</sup>LysM<sup>Cre</sup>+niacin: n=7). **F**, Effect of niacin on prostaglandin D<sub>2</sub> generation in the lungs from HySu-treated H-PGDS<sup>F/F</sup>LysM<sup>Cre</sup> and H-PGDS<sup>F/F</sup> mice. \*P<0.05 vs vehicle; #P<0.05 vs H-PGDS<sup>F/F</sup> (n=6 each). Data represent mean±SEM. Statistical significance was evaluated by Kruskal-Wallis tests followed by Dunn test. The exact P values are listed in Statistic Dataset in the Data Supplement. CSA indicates cross-sectional area.

the Data Supplement), and ultimately suppressed niacin-evoked PGD<sub>2</sub> production (Figure IXJ in the Data Supplement). Similarly, niacin receptor GPR109A was highly expressed in human macrophages differentiated from THP-1 (a human monocytic leukemia cell line) cells, and minimally expressed in human pulmonary artery endothelial cells, pulmonary arterial vascular smooth muscle cells, and lung fibroblasts (Figure XA in the Data Supplement). In addition, niacin markedly upregulated the H-PGDS mRNA level (Figure XB in the Data Supplement) and elevated PGD<sub>2</sub> production in THP-1 macrophages (Figure XC in the Data Supplement) but had no significant impact on L-PGDS gene expression (Figure XD in the Data Supplement). These results indicated that

niacin promoted PGD<sub>2</sub> release by GPR109A-mediated signaling pathway in macrophages.

## DISCUSSION

As an antihyperlipidemic agent, niacin has also been shown to reduce blood pressure and promote the resolution of inflammation. In this study, we demonstrated that niacin protected against experimentally induced PH through the inhibition of pulmonary vascular remodeling in rodents. Niacin-triggered pulmonary PGD<sub>2</sub> generation was mainly derived from H-PGDS, rather than L-PGDS, in macrophages in hypoxia-exposed mice. H-PGDS deficiency in macrophages, but not in mast cells, markedly

attenuated the protective effects of niacin against HySu-induced PH in mice, suggesting that niacin exerts beneficial effects on PAH through the induction of macrophage H-PGDS-derived PGD<sub>2</sub>.

PGD<sub>2</sub> was markedly elevated in patients with primary pulmonary hypertension and in rodent PH models.<sup>35</sup> Treatment with PGD<sub>2</sub> specifically prevented hypoxic pulmonary vasoconstriction, without affecting systemic pressures, and reversed induced pulmonary hypertension in newborn lambs,<sup>36,37</sup> suggesting a vital role for PGD<sub>2</sub> in the maintenance of pulmonary vascular homeostasis. Accordingly, we found that niacin evoked a great increase in PGD<sub>2</sub> production in lung tissues of PH mouse models and markedly attenuated PH in mice and rats. Systemic biosynthesis of PGD<sub>2</sub> occurs mainly through H-PGDS (about 90%), and only partially through L-PGDS in mice.<sup>38</sup> Consistently, we found that ablation of H-PGDS, but not L-PGDS, significantly suppressed PGD<sub>2</sub> production in the lungs of mice. Meanwhile, H-PGDS deficiency exacerbated HySu-induced PH in mice and abolished niacin's therapeutic effect on PH. Although deletion of L-PGDS, but not H-PGDS, elevated systemic blood pressure, this effect may be not directly associated with the suppression of systemic PGD<sub>2</sub> biosynthesis.<sup>38</sup> Furthermore, H-PGDS-derived PGD<sub>2</sub> is assumed to act mainly as an inflammatory mediator.<sup>39,40</sup> Compared with L-PGDS, deletion of H-PGDS significantly inhibited PGD<sub>2</sub> generation from infiltrated inflammatory cells in tumors.<sup>27</sup> The actual cell type responsible for PGD<sub>2</sub> release in response to niacin is complicated, and dermal Langerhans cells,<sup>41</sup> macrophages,<sup>34</sup> mast cells,<sup>15</sup> and platelets<sup>42</sup> have all been implicated in niacin-induced flushing. Consistent with these observations, we found that H-PGDS was expressed in both perivascular macrophages and mast cells in HySu-induced PH mice and patients with idiopathic PAH. Specific deletion of H-PGDS in macrophages, but not in mast cells, or H-PGDS<sup>-/-</sup> bone marrow transplantation abrogated niacin's therapeutic effect on HySu-induced PH in mice. Indeed, PGD<sub>2</sub> generated from H-PGDS in macrophages has been shown to mediate niacin's cardioprotective effects,<sup>23</sup> while mast cell-specific H-PGDS-derived PGD<sub>2</sub> governs the tumor microenvironment and food allergy.<sup>27,43</sup> Paradoxically, mast cell-depleted Kit<sup>W-sh/W-sh</sup> mice produced more PGD<sub>2</sub> in lung tissues in response to HySu exposure. This was probably due to increased macrophage-mediated pulmonary inflammation resolution through upregulation of H-PGDS in Kit<sup>W-sh/W-sh</sup> mice with ameliorated PH.<sup>44</sup> Thus, niacin may block PAH progression by promoting PGD<sub>2</sub> release from macrophages.

Pulmonary vascular remodeling is a key pathological feature of PAH, characterized by concentric pulmonary arterial wall thickening and intimal lesions due to vascular smooth muscle cell (VSMC) proliferation, migration, and hypertrophy.<sup>45</sup> PGD<sub>2</sub> has been shown to inhibit the growth of prostate,<sup>46</sup> colon,<sup>47</sup> and gastric cancer cells,<sup>48</sup> as well as inhibit platelet-derived growth factor-induced

proliferation of mouse VSMCs.<sup>49</sup> Accordingly, we found that niacin induced PGD<sub>2</sub> release from macrophages and ameliorated pulmonary vascular remodeling in PAH by suppressing pulmonary VSMC hypertrophy. PGD<sub>2</sub> exerts its biological functions via 2 G protein-coupled receptors, DP1 (PGD<sub>2</sub> receptor subtype 1) and DP2.<sup>50</sup> Notably, DP1, but not DP2, mediated niacin-induced vasodilation in mice and humans.<sup>24,51</sup> Again, blockade of DP1, but not DP2, abrogated niacin's beneficial effects in the management of inflammatory bowel disease and myocardial infarction in mice.<sup>22,23</sup> Indeed, we found that, like prostacyclin receptors,<sup>52</sup> DP1 is downregulated in PAs from rodents with induced PH and in patients with idiopathic PAH. DP1 activation also attenuated hypoxia-induced PA remodeling and pulmonary VSMC hypertrophy through PKA-mediated dissociation of raptor from the mTORC1 (mammalian target of rapamycin complex 1) complex.<sup>53</sup> Thus, the suppression of hypoxia-induced pulmonary vascular remodeling by niacin observed in the present study was likely due to PGD<sub>2</sub>/DP1 axis-mediated inhibition of pulmonary VSMC hypertrophy.

In summary, we demonstrated that niacin blocked the progression of HySu-induced PH in rodents through a reduction of vascular remodeling achieved by stimulating H-PGDS-dependent PGD<sub>2</sub> release from macrophages. Our results suggest that niacin may serve as a therapeutic option for patients with PAH.

## ARTICLE INFORMATION

Received February 6, 2020; revision received August 28, 2020; accepted September 9, 2020.

### Affiliations

Pharmacology and Tianjin Key Laboratory of Inflammatory Biology, Key Laboratory of Immune Microenvironment and Disease (Ministry of Education), School of Basic Medical Sciences, Tianjin Medical University, Tianjin, China (D.J., J.L., G.C., Y.Y., J.Z., Y. Shen). Cardiology, Zhongshan Hospital, Fudan University, Shanghai Institute of Cardiovascular Diseases, Shanghai, China (D.J., P.B.). Shanghai Institute of Nutrition and Health, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Shanghai, China (D.J., P.B., N.W., Q.Z., Y.H., Y.Y.). Cardiology, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China (N.W., Q.Z., A.L.). Cardiology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, China (J.L., Y.Y.). Cardiology, Cardiovascular Institute and Fuwai Hospital, Peking Union Medical College and Chinese Academy of Medical Science, Beijing, China (J.W.). Cardiology, Second Affiliated Hospital, College of Medicine, Zhejiang University, Zhejiang, China (H.C., C.W.). International Institute for Integrative Sleep Medicine, University of Tsukuba, Tsukuba City, Japan (M.L.). Pharmacology and Toxicology, Medical College of Georgia, Augusta University, Georgia, United States of America (Y. Su). Isotope Science Center, The University of Tokyo, Tokyo, Japan (Y.U.).

### Sources of Funding

This work was supported by the National Key R&D Program of China (2018YFA0800601, 2017YFC1307404, 2017YFC1307402), National Natural Science Foundation of China (81790623, 81525004, 91439204, 31771269, 81970540, and 81771513), Natural Science Foundation of Tianjin (17JCYB-JC40700, 18JCYBJC27300, 17JCYBJC26800), Japan Society for the Promotion of Science (JSPS), Grants-in-Aid for Scientific Research (KAKENHI) grant 16H01881 (Y. Urade), Young Elite Scientists Sponsorship Program by Tianjin, TJSQNTJ-2018-10 (J. Zhang). Y. Yu is a Fellow at the Jiangsu Collaborative Innovation Center for Cardiovascular Disease Translational Medicine.

### Disclosures

None.

## Supplemental Materials

Expanded Online Materials & Methods

Online Figure Legends and Figures I–X

References<sup>7,32,53–71</sup>

Statistic Data Set

Major Resources Table

Uncropped Gel Blots

## REFERENCES

- Lau EMT, Giannoulatou E, Celermajer DS, Humbert M. Epidemiology and treatment of pulmonary arterial hypertension. *Nat Rev Cardiol.* 2017;14:603–614. doi: 10.1038/nrcardio.2017.84
- Thompson AAR, Lawrie A. Targeting vascular remodeling to treat pulmonary arterial hypertension. *Trends Mol Med.* 2017;23:31–45. doi: 10.1016/j.molmed.2016.11.005
- Rabinovitch M, Guignabert C, Humbert M, Nicolls MR. Inflammation and immunity in the pathogenesis of pulmonary arterial hypertension. *Circ Res.* 2014;115:165–175. doi: 10.1161/CIRCRESAHA.113.301141
- Price LC, Wort SJ, Perros F, Dorfmüller P, Huertas A, Montani D, Cohen-Kaminsky S, Humbert M. Inflammation in pulmonary arterial hypertension. *Chest.* 2012;141:210–221. doi: 10.1378/chest.11-0793
- Hassoun PM, Mouthon L, Barberà JA, Eddahibi S, Flores SC, Grimminger F, Jones PL, Maitland ML, Michelakis ED, Morrell NW, et al. Inflammation, growth factors, and pulmonary vascular remodeling. *J Am Coll Cardiol.* 2009;54:S10–S19. doi: 10.1016/j.jacc.2009.04.006
- Savai R, Al-Tamari HM, Sedding D, Kojonazarov B, Muecke C, Teske R, Capecchi MR, Weissmann N, Grimminger F, Seeger W, et al. Pro-proliferative and inflammatory signaling converge on FoxO1 transcription factor in pulmonary hypertension. *Nat Med.* 2014;20:1289–1300. doi: 10.1038/nm.3695
- Lu A, Zuo C, He Y, Chen G, Piao L, Zhang J, Xiao B, Shen Y, Tang J, Kong D, et al. EP3 receptor deficiency attenuates pulmonary hypertension through suppression of Rho/TGF-β1 signaling. *J Clin Invest.* 2015;125:1228–1242. doi: 10.1172/JCI77656
- Chapman MJ, Redfern JS, McGovern ME, Giral P. Niacin and fibrates in atherosogenic dyslipidemia: pharmacotherapy to reduce cardiovascular risk. *Pharmacol Ther.* 2010;126:314–345. doi: 10.1016/j.pharmthera.2010.01.008
- Lavigne PM, Karas RH. The current state of niacin in cardiovascular disease prevention: a systematic review and meta-regression. *J Am Coll Cardiol.* 2013;61:440–446. doi: 10.1016/j.jacc.2012.10.030
- Wu BJ, Yan L, Charlton F, Witting P, Barter PJ, Rye KA. Evidence that niacin inhibits acute vascular inflammation and improves endothelial dysfunction independent of changes in plasma lipids. *Arterioscler Thromb Vasc Biol.* 2010;30:968–975. doi: 10.1161/ATVBAHA.109.201129
- Gadegbeku CA, Dhandayuthapani A, Shrayef MZ, Egan BM. Hemodynamic effects of nicotinic acid infusion in normotensive and hypertensive subjects. *Am J Hypertens.* 2003;16:67–71. doi: 10.1016/s0895-7061(02)03196-5
- Canner PL, Furberg CD, McGovern ME. Benefits of niacin in patients with versus without the metabolic syndrome and healed myocardial infarction (from the Coronary Drug Project). *Am J Cardiol.* 2006;97:477–479. doi: 10.1016/j.amjcard.2005.08.070
- The Coronary Drug Project Research Group. Clofibrate and niacin in coronary heart-disease. *JAMA.* 1975;231:360–381.
- Bays HE, MacCubbin D, Meehan AG, Kuznetsova O, Mitchel YB, Paolini JF. Blood pressure-lowering effects of extended-release niacin alone and extended-release niacin/laropiprant combination: a post hoc analysis of a 24-week, placebo-controlled trial in dyslipidemic patients. *Clin Ther.* 2009;31:115–122. doi: 10.1016/j.clinthera.2009.01.010
- Papalioidis D, Boucher W, Kempuraj D, Michaelian M, Wolfberg A, House M, Theoharides TC. Niacin-induced “flush” involves release of prostaglandin D2 from mast cells and serotonin from platelets: evidence from human cells in vitro and an animal model. *J Pharmacol Exp Ther.* 2008;327:665–672. doi: 10.1124/jpet.108.141333
- Meyers CD, Liu P, Kamanna VS, Kashyap ML. Nicotinic acid induces secretion of prostaglandin D2 in human macrophages: an in vitro model of the niacin flush. *Atherosclerosis.* 2007;192:253–258. doi: 10.1016/j.atherosclerosis.2006.07.014
- Seo MJ, Oh DK. Prostaglandin synthases: molecular characterization and involvement in prostaglandin biosynthesis. *Prog Lipid Res.* 2017;66:50–68. doi: 10.1016/j.plipres.2017.04.003
- Kuvit JT, Dave DM, Slaney KA, Mooney P, Patel AR, Kimmelstiel CD, Karas RH. Effects of extended-release niacin on lipoprotein particle size, distribution, and inflammatory markers in patients with coronary artery disease. *Am J Cardiol.* 2006;98:743–745. doi: 10.1016/j.amjcard.2006.04.011
- Godin AM, Ferreira WC, Rocha LT, Ferreira RG, Paiva AL, Merlo LA, Nascimento EB Jr, Bastos LF, Coelho MM. Nicotinic acid induces antinociceptive and anti-inflammatory effects in different experimental models. *Pharmacol Biochem Behav.* 2012;101:493–498. doi: 10.1016/j.pbb.2012.02.012
- Singh N, Gurav A, Sivaprakasam S, Brady E, Padia R, Shi H, Thangaraju M, Prasad PD, Manicassamy S, Munn DH, et al. Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. *Immunity.* 2014;40:128–139. doi: 10.1016/j.jimmuni.2013.12.007
- Kong D, Shen Y, Liu G, Zuo S, Ji Y, Lu A, Nakamura M, Lazarus M, Stratakis CA, Breyer RM, et al. PKA regulatory IIα subunit is essential for PGD2-mediated resolution of inflammation. *J Exp Med.* 2016;213:2209–2226. doi: 10.1084/jem.20160459
- Li J, Kong D, Wang Q, Wu W, Tang Y, Bai T, Guo L, Wei L, Zhang Q, Yu Y, et al. Niacin ameliorates ulcerative colitis via prostaglandin D2-mediated D prostanoid receptor 1 activation. *EMBO Mol Med.* 2017;9:571–588. doi: 10.15252/emmm.201606987
- Kong D, Li J, Shen Y, Liu G, Zuo S, Tao B, Ji Y, Lu A, Lazarus M, Breyer RM, et al. Niacin promotes cardiac healing after myocardial infarction through activation of the myeloid prostaglandin D2 receptor subtype 1. *J Pharmacol Exp Ther.* 2017;360:435–444. doi: 10.1124/jpet.116.238261
- Cheng K, Wu TJ, Wu KK, Sturino C, Metters K, Gottesdiener K, Wright SD, Wang Z, O’Neill G, Lai E, et al. Antagonism of the prostaglandin D2 receptor 1 suppresses nicotinic acid-induced vasodilation in mice and humans. *Proc Natl Acad Sci USA.* 2006;103:6682–6687. doi: 10.1073/pnas.0601574103
- Murata T, Aritake K, Tsubosaka Y, Maruyama T, Nakagawa T, Hori M, Hirai H, Nakamura M, Narumiya S, Urade Y, et al. Anti-inflammatory role of PGD2 in acute lung inflammation and therapeutic application of its signal enhancement. *Proc Natl Acad Sci USA.* 2013;110:5205–5210. doi: 10.1073/pnas.1218091110
- Thenappan T, Ormiston ML, Ryan JJ, Archer SL. Pulmonary arterial hypertension: pathogenesis and clinical management. *BMJ.* 2018;360:j5492. doi: 10.1136/bmj.j5492
- Murata T, Aritake K, Matsumoto S, Kamauchi S, Nakagawa T, Hori M, Momotani E, Urade Y, Ozaki H. Prostaglandin D2 is a mast cell-derived antiangiogenic factor in lung carcinoma. *Proc Natl Acad Sci U S A.* 2011;108:19802–19807. doi: 10.1073/pnas.1110011108
- Gandhi UH, Kaushal N, Ravindra KC, Hegde S, Nelson SM, Narayan V, Vunta H, Paulson RF, Prabhu KS. Selenoprotein-dependent up-regulation of hematopoietic prostaglandin D2 synthase in macrophages is mediated through the activation of peroxisome proliferator-activated receptor (PPAR) gamma. *J Biol Chem.* 2011;286:27471–27482. doi: 10.1074/jbc.M111.260547
- Tanaka K, Ogawa K, Sugamura K, Nakamura M, Takano S, Nagata K. Cutting edge: differential production of prostaglandin D2 by human helper T cell subsets. *J Immunol.* 2000;164:2277–2280. doi: 10.4049/jimmunol.164.5.2277
- Mahmud I, Ueda N, Yamaguchi H, Yamashita R, Yamamoto S, Kanaoka Y, Urade Y, Hayaishi O. Prostaglandin D synthase in human megakaryoblastic cells. *J Biol Chem.* 1997;272:28263–28266. doi: 10.1074/jbc.272.45.28263
- Dudeck A, Dudeck J, Scholten J, Petzold A, Surianarayanan S, Köhler A, Peschke K, Vöhringer D, Waskow C, Krieg T, et al. Mast cells are key promoters of contact allergy that mediate the adjuvant effects of haptens. *Immunity.* 2011;34:973–984. doi: 10.1016/j.jimmuni.2011.03.028
- Tono T, Tsujimura T, Koshimizu U, Kasugai T, Adachi S, Isozaki K, Nishikawa S, Morimoto M, Nishimura Y, Nomura S. c-kit Gene was not transcribed in cultured mast cells of mast cell-deficient Wsh/Wsh mice that have a normal number of erythrocytes and a normal c-kit coding region. *Blood.* 1992;80:1448–1453.
- Walters RW, Shukla AK, Kovacs JJ, Violin JD, DeWire SM, Lam CM, Chen JR, Muehlbauer MJ, Whalen EJ, Lefkowitz RJ. beta-Arrestin1 mediates nicotinic acid-induced flushing, but not its antilipolytic effect, in mice. *J Clin Invest.* 2009;119:1312–1321. doi: 10.1172/JCI36806
- Benyo Z, Gille A, Kero J, Csiky M, Suchánková MC, Nüsing RM, Moers A, Pfeffer K, Offermanns S. GPR109A (PUMA-G/HM74A) mediates nicotinic acid-induced flushing. *J Clin Invest.* 2005;115:3634–3640. doi: 10.1172/JCI23626
- Robbins IM, Barst RJ, Rubin LJ, Gaine SP, Price PV, Morrow JD, Christman BW. Increased levels of prostaglandin D(2) suggest macrophage activation in patients with primary pulmonary hypertension. *Chest.* 2001;120:1639–1644. doi: 10.1378/chest.120.5.1639

36. Soifer SJ, Morin FC 3<sup>rd</sup>, Heymann MA. Prostaglandin D2 reverses induced pulmonary hypertension in the newborn lamb. *J Pediatr.* 1982;100:458–463. doi: 10.1016/s0022-3476(82)80460-5
37. Philips JB 3<sup>rd</sup>, Lyrene RK, McDevitt M, Perlis W, Satterwhite C, Cassady G. Prostaglandin D2 inhibits hypoxic pulmonary vasoconstriction in neonatal lambs. *J Appl Physiol Respir Environ Exerc Physiol.* 1983;54:1585–1589. doi: 10.1152/jappl.1983.54.6.1585
38. Song WL, Ricciotti E, Liang X, Grosser T, Grant GR, Fitzgerald GA. Lipocalin-like prostaglandin D synthase but not hemopoietic prostaglandin D synthase deletion causes hypertension and accelerates thrombogenesis in mice. *J Pharmacol Exp Ther.* 2018;367:425–432. doi: 10.1124/jpet.118.250936
39. Kostenis E, Ulven T. Emerging roles of DP and CRTH2 in allergic inflammation. *Trends Mol Med.* 2006;12:148–158. doi: 10.1016/j.molmed.2006.02.005
40. Kanaoka Y, Urade Y. Hematopoietic prostaglandin D synthase. *Prostaglandins Leukot Essent Fatty Acids.* 2003;69:163–167. doi: 10.1016/s0952-3278(03)00077-2
41. Benyó Z, Gilje A, Bennett CL, Clausen BE, Offermanns S. Nicotinic acid-induced flushing is mediated by activation of epidermal langerhans cells. *Mol Pharmacol.* 2006;70:1844–1849. doi: 10.1124/mol.106.030833
42. Song WL, Stubbe J, Ricciotti E, Alamuddin N, Ibrahim S, Crichton I, Prempeh M, Lawson JA, Wilensky RL, Rasmussen LM, et al. Niacin and biosynthesis of PGD<sub>2</sub> by platelet COX-1 in mice and humans. *J Clin Invest.* 2012;122:1459–1468. doi: 10.1172/JCI59262
43. Nakamura T, Maeda S, Horiguchi K, Maehara T, Aritake K, Choi BI, Iwakura Y, Urade Y, Murata T. PGD<sub>2</sub> deficiency exacerbates food antigen-induced mast cell hyperplasia. *Nat Commun.* 2015;6:7514. doi: 10.1038/ncomms8514
44. Rajakarier R, Hilliard M, Lawrence T, Trivedi S, Colville-Nash P, Bellinger G, Fitzgerald D, Yaqoob MM, Gilroy DW. Hematopoietic prostaglandin D2 synthase controls the onset and resolution of acute inflammation through PGD<sub>2</sub> and 15-deoxyDelta12 14 PGJ<sub>2</sub>. *Proc Natl Acad Sci USA.* 2007;104:20979–20984. doi: 10.1073/pnas.0707394104
45. Stenmark KR, Fagan KA, Frid MG. Hypoxia-induced pulmonary vascular remodeling: cellular and molecular mechanisms. *Circ Res.* 2006;99:675–691. doi: 10.1161/RES.0000243584.451453f
46. Kim J, Yang P, Suraokar M, Sabichi AL, Llansa ND, Mendoza G, Subbarayan V, Logothetis CJ, Newman RA, Lippman SM, et al. Suppression of prostate tumor cell growth by stromal cell prostaglandin D synthase-derived products. *Cancer Res.* 2005;65:6189–6198. doi: 10.1158/0008-5472.CAN-04-4439
47. Iwanaga K, Nakamura T, Maeda S, Aritake K, Hori M, Urade Y, Ozaki H, Murata T. Mast cell-derived prostaglandin D2 inhibits colitis and colitis-associated colon cancer in mice. *Cancer Res.* 2014;74:3011–3019. doi: 10.1158/0008-5472.CAN-13-2792
48. Zhang B, Bie Q, Wu P, Zhang J, You B, Shi H, Qian H, Xu W. PGD<sub>2</sub>/PTGDR2 signaling restricts the self-renewal and tumorigenesis of gastric cancer. *Stem Cells.* 2018;36:990–1003. doi: 10.1002/stem.2821
49. Fujino T, Yuhki K, Yamada T, Hara A, Takahata O, Okada Y, Xiao CY, Ma H, Karibe H, Iwashima Y, et al. Effects of the prostanoids on the proliferation or hypertrophy of cultured murine aortic smooth muscle cells. *Br J Pharmacol.* 2002;136:530–539. doi: 10.1038/sj.bjp.0704749
50. Pettipher R. The roles of the prostaglandin D<sub>2</sub> receptors DP(1) and CRTH2 in promoting allergic responses. *Br J Pharmacol.* 2008;153(Suppl 1):S191–S199. doi: 10.1038/sj.bjp.0707488
51. Walch L, Labat C, Gascard JP, de Montpreville V, Brink C, Norel X. Prostanoid receptors involved in the relaxation of human pulmonary vessels. *Br J Pharmacol.* 1999;126:859–866. doi: 10.1038/sj.bjp.0702393
52. Falcetti E, Hall SM, Phillips PG, Patel J, Morrell NW, Haworth SG, Clapp LH. Smooth muscle proliferation and role of the prostacyclin (IP) receptor in idiopathic pulmonary arterial hypertension. *Am J Respir Crit Care Med.* 2010;182:1161–1170. doi: 10.1164/rccm.201001-0011OC
53. He Y, Zuo C, Jia D, Bai P, Kong D, Chen D, Liu G, Li J, Wang Y, Chen G, et al. Loss of DP1 aggravates vascular remodeling in pulmonary arterial hypertension via mTORC1 signaling. *Am J Respir Crit Care Med.* 2020;201:1263–1276. doi: 10.1164/rccm.201911-2137OC
54. Eguchi N, Minami T, Shirafuji N, Kanaoka Y, Tanaka T, Nagata A, Yoshida N, Urade Y, Ito S, Hayashi O. Lack of tactile pain (allodynia) in lipocalin-type prostaglandin D synthase-deficient mice. *Proc Natl Acad Sci USA.* 1999;96:726–730. doi: 10.1073/pnas.96.2.726
55. Nakamura T, Fujiwara Y, Yamada R, Fujii W, Hamabata T, Lee MY, Maeda S, Aritake K, Roers A, Sessa WC, et al. Mast cell-derived prostaglandin D2 attenuates anaphylactic reactions in mice. *J Allergy Clin Immunol.* 2017;140:630–632.e9. doi: 10.1016/j.jaci.2017.02.030
56. White K, Dempsey Y, Nilsen M, Wright AF, Loughlin L, MacLean MR. The serotonin transporter, gender, and 17 $\beta$  oestradiol in the development of pulmonary arterial hypertension. *Cardiovasc Res.* 2011;90:373–382. doi: 10.1093/cvr/cvq408
57. Jia D, He Y, Zhu Q, Liu H, Zuo C, Chen G, Yu Y, Lu A. RAGE-mediated extracellular matrix proteins accumulation exacerbates HySu-induced pulmonary hypertension. *Cardiovasc Res.* 2017;113:586–597. doi: 10.1093/cvr/cvx051
58. van der Hoorn JW, de Haan W, Berbée JF, Havekes LM, Jukema JW, Rensen PC, Princen HM. Niacin increases HDL by reducing hepatic expression and plasma levels of cholesteryl ester transfer protein in APOE\*3Leiden CETP mice. *Arterioscler Thromb Vasc Biol.* 2008;28:2016–2022. doi: 10.1161/ATVBAHA.108.171363
59. Nikam VS, Schermuly RT, Dumitrascu R, Weissmann N, Kwapiszewska G, Morrell N, Klepetko W, Fink L, Seeger W, Voswinckel R. Treprostinil inhibits the recruitment of bone marrow-derived circulating fibrocytes in chronic hypoxic pulmonary hypertension. *Eur Respir J.* 2010;36:1302–1314. doi: 10.1183/09031936.00028009
60. Farkas D, Thompson AAR, Bhagwani AR, Hultman S, Ji H, Kotha N, Farr G, Arnold ND, Braithwaite A, Casbolt H, et al. Toll-like receptor 3 is a therapeutic target for pulmonary hypertension. *Am J Respir Crit Care Med.* 2019;199:199–210. doi: 10.1164/rccm.201707-1370OC
61. Calvier L, Boucher P, Herz J, Hansmann G. LRP1 deficiency in vascular SMC leads to pulmonary arterial hypertension that is reversed by PPAR $\gamma$  activation. *Circ Res.* 2019;124:1778–1785. doi: 10.1161/CIRCRESAHA.119.315088
62. Hameed AG, Arnold ND, Chamberlain J, Pickworth JA, Paiva C, Dawson S, Cross S, Long L, Zhao L, Morrell NW, et al. Inhibition of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) reverses experimental pulmonary hypertension. *J Exp Med.* 2012;209:1919–1935. doi: 10.1084/jem.20112716
63. Frederburgh LE, Liang OD, Macias AA, Polte TR, Liu X, Riascos DF, Chung SW, Schissel SL, Ingber DE, Mitsialis SA, et al. Absence of cyclooxygenase-2 exacerbates hypoxia-induced pulmonary hypertension and enhances contractility of vascular smooth muscle cells. *Circulation.* 2008;117:2114–2122. doi: 10.1161/CIRCULATIONAHA.107.716241
64. Chen G, Zuo S, Tang J, Zuo C, Jia D, Liu Q, Liu G, Zhu Q, Wang Y, Zhang J, et al. Inhibition of CRTH2-mediated Th2 activation attenuates pulmonary hypertension in mice. *J Exp Med.* 2018;215:2175–2195. doi: 10.1084/jem.20171767
65. Khan OM, Akula MK, Skålen K, Karlsson C, Ståhlman M, Young SG, Borén J, Bergé MO. Targeting GGTase-I activates RHOA, increases macrophage reverse cholesterol transport, and reduces atherosclerosis in mice. *Circulation.* 2013;127:782–790. doi: 10.1161/CIRCULATIONAHA.112.000588
66. Yuan K, Shamskhoo EA, Orcholski ME, Nathan A, Reddy S, Honda H, Mani V, Zeng Y, Ozen MO, Wang L, et al. Loss of endothelium-derived Wnt5a is associated with reduced pericyte recruitment and small vessel loss in pulmonary arterial hypertension. *Circulation.* 2019;139:1710–1724. doi: 10.1161/CIRCULATIONAHA.118.037642
67. Tager AM, Kradin RL, LaCamera P, Bercury SD, Campanella GS, Leary CP, Polosukhin V, Zhao LH, Sakamoto H, Blackwell TS, et al. Inhibition of pulmonary fibrosis by the chemokine IP-10/CXCL10. *Am J Respir Cell Mol Biol.* 2004;31:395–404. doi: 10.1165/rccb.2004-0175OC
68. Tager AM, LaCamera P, Shea BS, Campanella GS, Selman M, Zhao Z, Polosukhin V, Wain J, Karimi-Shah BA, Kim ND, et al. The lysophosphatidic acid receptor LPA1 links pulmonary fibrosis to lung injury by mediating fibroblast recruitment and vascular leak. *Nat Med.* 2008;14:45–54. doi: 10.1038/nm1685
69. Umezuri K, Koga JI, Matoba T, Katsuki S, Wang L, Hasuzawa N, Nomura M, Tsutsui H, Egashira K. Macrophage (Drp1) dynamin-related protein 1 accelerates intimal thickening after vascular injury. *Arterioscler Thromb Vasc Biol.* 2020;40:e214–e226.
70. Kass D, Bridges RS, Borczuk A, Greenberg S. Methionine aminopeptidase-2 as a selective target of myofibroblasts in pulmonary fibrosis. *Am J Respir Cell Mol Biol.* 2007;37:193–201. doi: 10.1165/rccb.2006-0352OC
71. Savai R, Pullamsetti SS, Kolbe J, Bieniek E, Voswinckel R, Fink L, Scheid A, Ritter C, Dahal BK, Vafer A, et al. Immune and inflammatory cell involvement in the pathology of idiopathic pulmonary arterial hypertension. *Am J Respir Crit Care Med.* 2012;186:897–908. doi: 10.1164/rccm.201202-0335OC