

# Omega-3 fatty acid epoxides are autocrine mediators that control the magnitude of IgE-mediated mast cell activation

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Critical to the function of mast cells in immune responses including allergy is their production of lipid mediators, among which only omega-6 ( $\omega$ -6) arachidonate-derived eicosanoids have been well characterized. Here, by employing comprehensive lipidomics, we identify omega-3 ( $\omega$ -3) fatty acid epoxides as new mast cell-derived lipid mediators and show that they are produced by PAF-AH2, an oxidized-phospholipid-selective phospholipase A2. Genetic or pharmacological deletion of PAF-AH2 reduced the steady-state production of  $\omega$ -3 epoxides, leading to attenuated mast cell activation and anaphylaxis following Fc $\epsilon$ RI cross-linking. Mechanistically, the  $\omega$ -3 epoxides promote IgE-mediated activation of mast cells by downregulating Srcin1, a Src-inhibitory protein that counteracts Fc $\epsilon$ RI signaling, through a pathway involving PPAR $\gamma$ . Thus, the PAF-AH2- $\omega$ -3 epoxide-Srcin1 axis presents new potential drug targets for allergic diseases.

Allergic diseases such as atopic dermatitis and asthma are becoming very common in developed countries<sup>1</sup>. Allergy is associated with increased serum IgE levels and mast cell activation. Association of an antigen with IgE on the mast cell surface causes cross-linking of the high-affinity IgE receptor (Fc $\epsilon$ RI), leading to exocytosis of granule-associated mediators and proteases (degranulation) as well as new synthesis of cytokines and lipid mediators<sup>2</sup>. Therefore, uncovering molecular mechanisms that regulate mast cell activation could provide crucial insights into the pathophysiology of mast cell-associated diseases.

Mast cells produce lipid mediators derived from arachidonic acid (AA; an  $\omega$ -6 fatty acid) called eicosanoids, including prostaglandins (PGs) and leukotrienes (LTs), as effector molecules that act in autocrine and paracrine fashions. Recently, oxygenated products derived from  $\omega$ -3 fatty acids, primarily eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have attracted attention as a new class of pro-resolving lipid mediators including resolvins and protectins<sup>3</sup>. In addition,  $\omega$ -3 fatty acids are reported to have the capacity to attenuate allergic symptoms<sup>4,5</sup>. Phospholipase A2 (PLA2) enzymes lie upstream of the  $\omega$ -6 and  $\omega$ -3 lipid mediators and supply precursor fatty acids from membrane phospholipids<sup>6</sup>. However, the biological importance of  $\omega$ -3 fatty acid metabolites and the PLA2 subtype(s) responsible for their mobilization in mast cells remain poorly understood.

Here we performed a comprehensive lipidomics analysis of oxygenated metabolites of AA, EPA and DHA produced by mast cells and identified particular epoxyoxygenated EPA and DHA metabolites ( $\omega$ -3 epoxides) as a new class of lipid mediators that unexpectedly enhanced mast cell activation and anaphylaxis. The mechanism involved Src-inhibitory protein downstream of peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ), a lipid-sensing nuclear receptor that negatively regulates mast cell activation<sup>7</sup>. We also identified type II platelet-activating factor acetylhydrolase (PAF-AH2) as an  $\omega$ -3 epoxide-producing PLA2 subtype, and this enzyme could be a druggable target to combat allergic diseases.

## RESULTS

### PAF-AH2 mediates IgE-antigen-independent release of oxidized $\omega$ -3 fatty acids

To obtain a comprehensive profile of the lipid mediators produced by mast cells, we stimulated IL-3-dependent mouse bone marrow-derived mast cells (BMMCs) with IgE and antigen (dinitrophenylated human serum albumin (DNP-HSA)) and analyzed oxidized metabolites of AA, EPA and DHA in the culture supernatant by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The stimulated BMMCs released AA and its metabolites

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(Supplementary Fig. 1a), as previously reported<sup>2</sup>. Antigen stimulation also increased the release of EPA and its metabolites, but not that of DHA and its metabolites. As expected from previous studies<sup>8–10</sup>, the antigen-induced release of AA and EPA together with their metabolites was virtually eliminated in *Pla2g4a*<sup>−/−</sup> BMMCs (Supplementary Fig. 1a), indicating that the release of these lipids depends on group IVA cytosolic PLA2 (cPLA2 $\alpha$ ; encoded by *Pla2g4a*).

We also measured oxidized metabolites of AA, EPA and DHA in the conditioned medium of BMMCs cultured for 4 d without IgE–antigen stimulation. Although production of most AA-derived prostanoids was very low or undetectable, substantial levels of EPA- and DHA-derived metabolites, such as 17,18-epoxyeicosatetraenoic acid (17,18-EpETE) and 19,20-epoxydocosapentaenoic acid (19,20-EpDPE), were detected in the medium (Fig. 1a and Supplementary Fig. 1b). Production of these metabolites was not further augmented by IgE–antigen stimulation, even after 4 d (Supplementary Fig. 1c). Thus, mast cells spontaneously and preferentially produced oxygenated  $\omega$ -3 fatty acid metabolites.

Among the known PLA2 subtypes tested, cPLA2 $\alpha$  (encoded by *Pla2g4a*), group VIB Ca<sup>2+</sup>-independent PLA2 (iPLA2 $\gamma$ ; encoded by *Pnpla8*) and PAF-AH2 (group VIIB PLA2; encoded by *Pafah2*) were expressed at much higher levels than other PLA2 enzymes in BMMCs (Fig. 1b). *Pla2g4a*<sup>−/−</sup> and *Pnpla8*<sup>−/−</sup> BMMCs produced EPA and DHA metabolites similar to those produced by wild-type BMMCs, whereas production of these metabolites was much lower in *Pafah2*<sup>−/−</sup> BMMCs (Fig. 1c and Supplementary Fig. 1b). These results suggest that PAF-AH2 is responsible for the IgE–antigen-independent constitutive production of oxygenated EPA and DHA metabolites by mast cells.

### Antigen-induced anaphylaxis is impaired in *Pafah2*<sup>−/−</sup> mice

Antibody against PAF-AH2 (ref. 11) immunohistochemically stained toluidine blue–positive dermal mast cells in wild-type but not *Pafah2*<sup>−/−</sup> mice (Fig. 2a), confirming the expression of PAF-AH2 in mast cells. Mast cell–dependent passive cutaneous anaphylaxis (PCA) as monitored by ear edema (Fig. 2b–d) and passive systemic anaphylaxis (PSA) as monitored by elevation of serum histamine levels and temporary decrease in rectal temperature (Fig. 2e,f) were markedly attenuated in *Pafah2*<sup>−/−</sup> mice compared to wild-type mice on a C57BL/6 background. The reduced PCA response was also evident in *Pafah2*<sup>−/−</sup> BALB/c mice (Supplementary Fig. 2a–c). Consistent with these findings, the number of degranulated skin mast cells was lower in *Pafah2*<sup>−/−</sup> mice than in wild-type mice upon antigen challenge, although the total mast cell numbers in the two genotypes were indistinguishable (Supplementary Fig. 2d–f). Expression levels of mast cell markers in the ear, stomach and small intestine were comparable between the two genotypes (Supplementary Fig. 2g–i), suggesting that proliferation and maturation of mast cells *in vivo* were not affected by PAF-AH2 deficiency. In contrast, antigen-independent anaphylaxis elicited by compound 48/80 was intact in *Pafah2*<sup>−/−</sup> mice (Fig. 2g–i), suggesting that the effect of PAF-AH2 ablation might be specific for Fc $\epsilon$ RI-dependent activation.

Wild-type and *Pafah2*<sup>−/−</sup> BMMCs were grafted intradermally into the ears of mast cell–deficient *Kit*<sup>W-sh/W-sh</sup> mice, which carry a mutation in the *Kit* locus encoding stem cell factor receptor. On transmission electron microscopy, *Pafah2*<sup>−/−</sup> BMMCs had apparently normal ultrastructure (Supplementary Fig. 2j). Cell-surface expression of Fc $\epsilon$ RI and c-Kit, expression of mast cell markers including *Hdc*, *Kit*, *Mcpt9*, *Mitf* and *Cpa3*, and histamine content were also normal in *Pafah2*<sup>−/−</sup> BMMCs (Supplementary Fig. 2k–n). Forty days after engraftment, the distribution and numbers of ear skin mast

cells (Supplementary Fig. 2o,p) and the expression of the mast cell marker *Mcpt4* (Supplementary Fig. 2q) were comparable between mice engrafted with wild-type and *Pafah2*<sup>−/−</sup> BMMCs. Reconstituting *Kit*<sup>W-sh/W-sh</sup> mice with wild-type BMMCs restored antigen-induced anaphylaxis as expected, whereas reconstituting *Kit*<sup>W-sh/W-sh</sup> mice with *Pafah2*<sup>−/−</sup> BMMCs only partially restored it (Fig. 2j–l). These results suggest that the PAF-AH2 intrinsically expressed in mast cells contributes to the antigen-dependent activation but not the proliferation or maturation of mast cells *in vivo*.

### Initial step of antigen-induced signaling is impaired in *Pafah2*<sup>−/−</sup> mast cells

Next, we examined which steps of antigen-induced activation were affected in *Pafah2*<sup>−/−</sup> BMMCs. Antigen-induced exocytosis of  $\beta$ -hexosaminidase ( $\beta$ -HEX) and histamine, production of eicosanoids (PGD<sub>2</sub> and LTC<sub>4</sub>) and secretion of cytokines (IL-6 and tumor necrosis factor (TNF)- $\alpha$ ) were all reduced in *Pafah2*<sup>−/−</sup> BMMCs as compared with wild-type BMMCs (Fig. 3a–f). In contrast, antigen-independent release of  $\beta$ -HEX and histamine elicited by ATP was intact in *Pafah2*<sup>−/−</sup> BMMCs (Supplementary Fig. 3a,b), implying again that PAF-AH2 ablation selectively attenuates Fc $\epsilon$ RI-dependent activation. Antigen treatment activates two Fc $\epsilon$ RI-proximal kinases, Lyn and Fyn; Lyn phosphorylates Fc $\epsilon$ RI leading to activation of the central kinase Syk<sup>12</sup>, while Fyn phosphorylates the adaptor protein Gab2 (ref. 13). Notably, antigen-induced phosphorylation of Fc $\epsilon$ RI, Syk and Gab2, which peaked at 2 min after antigen treatment, was reduced in *Pafah2*<sup>−/−</sup> BMMCs relative to wild-type BMMCs (Fig. 3g–i). Furthermore, antigen-induced activation of Lyn and Fyn was also reduced in *Pafah2*<sup>−/−</sup> BMMCs as compared with wild-type BMMCs (Fig. 3j–m), suggesting that Fc $\epsilon$ RI-proximal signaling is impaired in *Pafah2*<sup>−/−</sup> BMMCs. The activities of two other Src-family kinases, Fgr and Hck<sup>14–16</sup>, were comparable in the two genotypes (Supplementary Fig. 3c).

Retroviral expression of native PAF-AH2 rescued both antigen-dependent degranulation and phosphorylation of Syk and Gab2 in *Pafah2*<sup>−/−</sup> BMMCs, whereas the catalytically inactive Ser234Cys mutant<sup>17</sup> failed to recover these responses (Fig. 3n–p). Thus, PAF-AH2 influenced Fc $\epsilon$ RI-proximal signaling in a manner dependent on its catalytic activity.

### $\omega$ -3 epoxides restore antigen-induced signaling in *Pafah2*<sup>−/−</sup> mast cells

To identify the PAF-AH2-driven lipid products, lipids extracted from the conditioned media of *Pafah2*<sup>−/−</sup> BMMCs and *Pafah2*<sup>−/−</sup> BMMCs transduced to express native PAF-AH2 or its inactive mutant Ser234Cys were added to the culture of *Pafah2*<sup>−/−</sup> BMMCs (Fig. 4a). The free fatty acid fraction obtained from *Pafah2*<sup>−/−</sup> BMMCs expressing native PAF-AH2 rescued the impaired antigen-induced degranulation in *Pafah2*<sup>−/−</sup> BMMCs, whereas the fractions obtained from *Pafah2*<sup>−/−</sup> BMMCs and *Pafah2*<sup>−/−</sup> BMMCs reconstituted with PAF-AH2 Ser234Cys failed to do so (Fig. 4b and Supplementary Fig. 4a), suggesting that some fatty acid metabolites released by PAF-AH2 are responsible for proper antigen-dependent mast cell activation.

Given that the levels of several monooxygenated EPA and DHA metabolites were reduced in *Pafah2*<sup>−/−</sup> BMMCs (Fig. 1c), we speculated that some of these could rescue degranulation of *Pafah2*<sup>−/−</sup> BMMCs. These EPA and DHA metabolites included  $\omega$ -3 monohydroxides (18-, 19- and 20-hydroxyepoxyeicosatetraenoic acids (HEPEs) and 20-, 21- and 22-hydroxydocosahexaenoic acids (HDoHEs)) and  $\omega$ -3 epoxides (17,18-EpETE and 19,20-EpDPE) (Supplementary Fig. 4b). When mixtures of synthetic  $\omega$ -3 metabolites analogous to

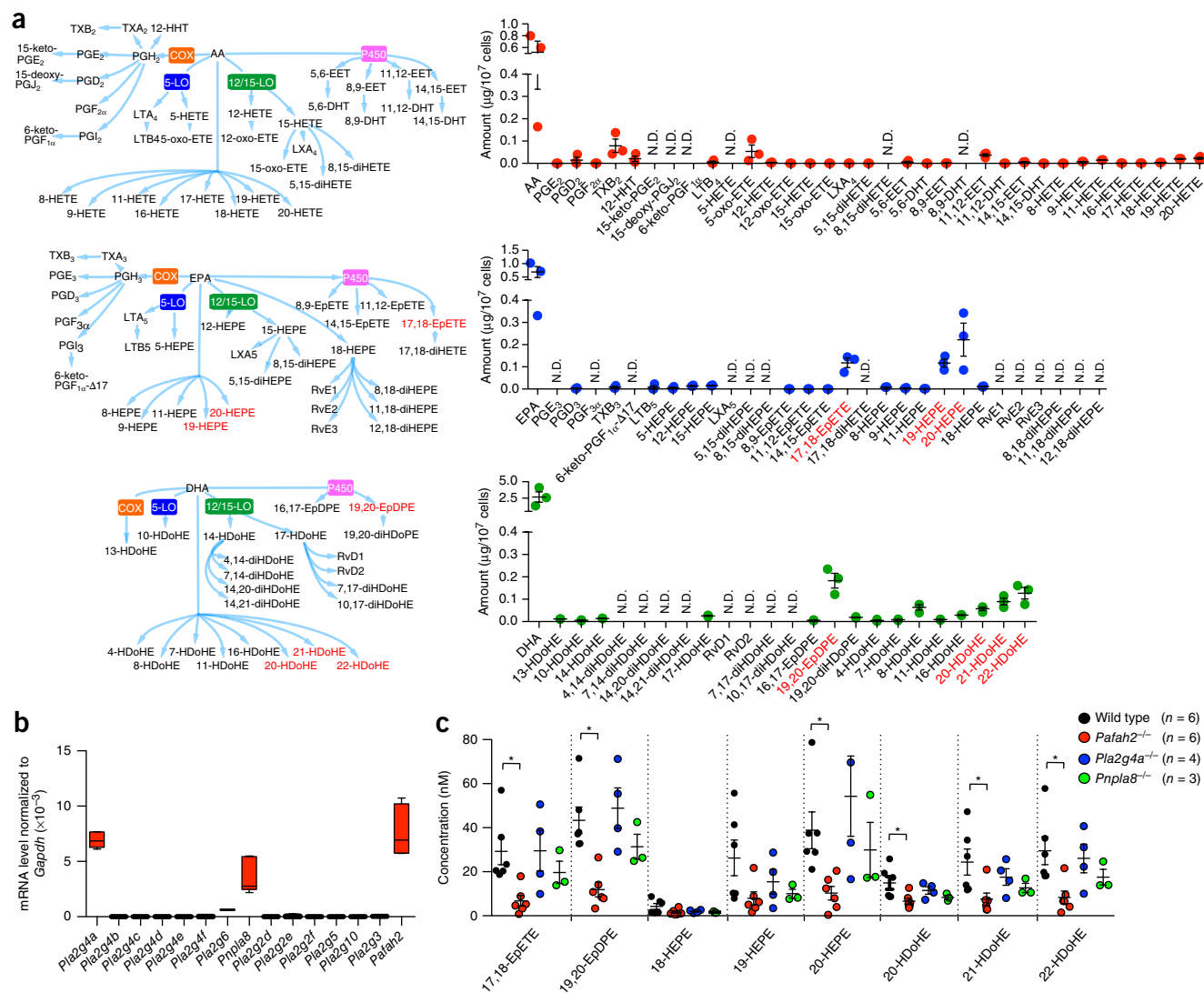


**Figure 1** PAF-AH2 mediates spontaneous release of oxidized  $\omega$ -3 fatty acids from mast cells. **(a)** The metabolic pathways of AA-, EPA- and DHA-derived oxidized fatty acids (left) and LC-MS/MS-based lipidomics of the conditioned medium of C57BL/6-derived BMMCs cultured for 4 d in the absence of IgE-antigen (Ag) stimulation (right;  $n = 3$  cultures from 3 mice). TX, thromboxane; HHT, hydroxyheptadecatrienoic acid; LX, lipoxin; EET, epoxyeicosatrienoic acid; HETE, hydroxyeicosatetraenoic acid; EpETE, epoxyeicosatetraenoic acid; HEPE, hydroxyeicosapentaenoic acid; diHETE, dihydroxyeicosatetraenoic acid; EpDPE, epoxydocosapentaenoic acid; HDoHE, hydroxydocosahexaenoic acid; diHDoHE, dihydroxy docosahexaenoic acid; Rv, resolvin. Values (per  $10^7$  cells) are means  $\pm$  s.e.m. N.D., not detectable. The EPA and DHA metabolites whose amounts are  $>0.5 \mu\text{g}/10^7$  cells are shown in red. **(b)** Real-time PCR of various PLA2 mRNAs in wild-type BMMCs ( $n = 3$  cultures from 3 mice). Center lines indicate the median, box limits represent the upper and lower quartiles, and whiskers extend to the minimum and maximum values. **(c)** LC-MS/MS-based lipidomics of BMMC conditioned medium.  $n$  represents the number of mice. See also **Supplementary Figure 1b**. Values are means  $\pm$  s.e.m. In **c**, data were analyzed by one-way ANOVA with Tukey's *post hoc* test:  $*P < 0.05$ . Data were from one set of experiments (**a,b**) or were compiled from two sets of experiments (**c**).

those detected in the conditioned medium of wild-type BMMCs were each added to *Pafah2*<sup>-/-</sup> BMMCs, the  $\omega$ -3 epoxides, but not the  $\omega$ -3 monohydroxides, restored antigen-induced degranulation of these BMMCs (**Fig. 4c**). Full restoration by addition of 17,18-EpETE or 19,20-EpDPE was achieved at 300 nM (**Fig. 4d** and **Supplementary Fig. 4c**), a concentration four times higher than the combined concentration for these metabolites in the conditioned medium (black dots in **Fig. 1c**). As these  $\omega$ -3 epoxides function intracellularly (see below), this concentration of exogenous  $\omega$ -3 epoxides might have been needed to generate the full activity. In contrast, the  $\omega$ -3 epoxides did not further enhance degranulation in wild-type BMMCs. The reduced phosphorylation of Fc $\epsilon$ RI, Syk and Gab2 in *Pafah2*<sup>-/-</sup> BMMCs was

also restored by 17,18-EpETE (**Fig. 4e** and **Supplementary Fig. 4d**). Fatty acid epoxides can be converted to dihydrodiols by soluble epoxy hydrolase (sEH)<sup>18</sup>. However, neither 17,18-diHETE nor 19,20-diHDoPE, which are the putative sEH-hydrolyzed products of 17,18-EpETE and 19,20-EpDPE, respectively, restored the impaired degranulation in *Pafah2*<sup>-/-</sup> BMMCs (**Fig. 4d**).

When injected *in vivo*, both  $\omega$ -3 epoxides restored antigen-induced anaphylaxis in *Pafah2*<sup>-/-</sup> mice to the level observed in wild-type mice (**Fig. 4f,g**). Consistent with this observation, treatment of *Pafah2*<sup>-/-</sup> mice with 17,18-EpETE increased the number of degranulated dermal mast cells after antigen challenge, without affecting the total number of mast cells (**Supplementary Fig. 4e,f**), whereas the

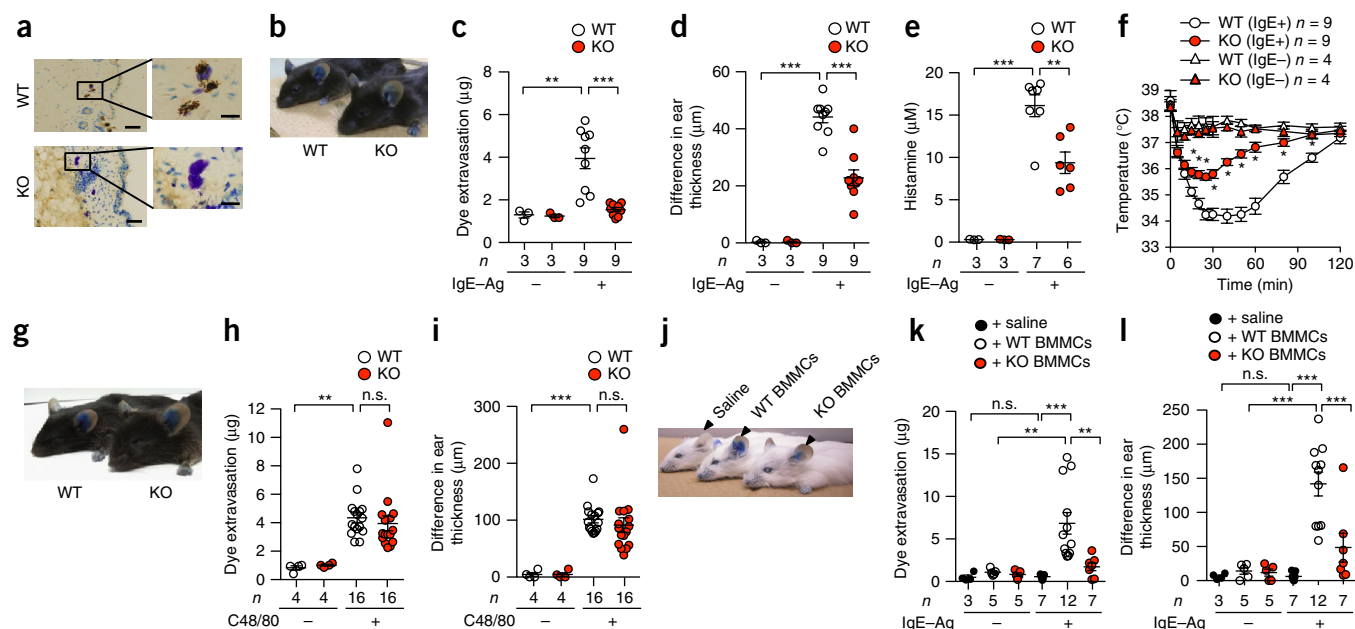


**Figure 2** Impaired antigen-induced anaphylaxis in *Pafah2*<sup>-/-</sup> mice. (a) Dorsal skin sections from wild-type (WT) or *Pafah2*<sup>-/-</sup> (KO) mice were stained with antibody against PAF-AH2 followed by counterstaining with toluidine blue (scale bars, 50  $\mu$ m). (b–d) PCA in WT and *Pafah2*<sup>-/-</sup> mice. (b) A representative photo is shown. Extravasation of Evans blue in the ears as a marker of vascular permeability (c) and differences in ear thickness (d) before and after antigen stimulation were evaluated. (e,f) Antigen-induced PSA in wild-type and *Pafah2*<sup>-/-</sup> mice after challenge with 500  $\mu$ g of antigen. Serum histamine concentrations as quantified by ELISA (e) and rectal temperatures (f) were evaluated. In f, \* $P$  < 0.05, WT versus KO after antigen challenge for the indicated periods determined by unpaired two-tailed Student's *t* test. (g–i) Wild-type and *Pafah2*<sup>-/-</sup> mice were challenged with 100 ng of compound 48/80 for 30 min or left untreated. (g) A representative photo is shown. Extravasation of Evans blue in the ears (h) and differences in ear thickness (i) were evaluated. (j–l) Antigen-induced PCA of *Kit*<sup>W-sh/W-sh</sup> mice and *Kit*<sup>W-sh/W-sh</sup> mice intradermally reconstituted with wild-type or *Pafah2*<sup>-/-</sup> BMDCs. (j) A representative photo of ear edema. Extravasation of Evans blue (k) and differences in ear thickness (l) were evaluated. All values are means  $\pm$  s.e.m. Data were analyzed by one-way ANOVA with Tukey's *post hoc* test in c–e, h, i, k and l: \* $P$  < 0.05; \*\* $P$  < 0.01; \*\*\* $P$  < 0.001; n.s., not significant ( $P$  > 0.05). Data were from one set of experiments (e,f,h,i,k,l) or were compiled from three sets of experiments (c,d). Images in a, b, g and j are representative of three experiments.

hydrolytic products 17,18-diHETE and 19,20-diHDoPE were inactive (Fig. 4f,g). Thus, the  $\omega$ -3 epoxides are likely candidates for the PAF-AH2-driven lipid mediators responsible for antigen-induced activation of mast cells.

#### PAF-AH2 releases $\omega$ -3 epoxides from membrane phospholipids

Because PAF-AH2 hydrolyzes phospholipids with oxidized fatty acyl chains, such as F<sub>2</sub>-isoprostane, in marked preference to those with long fatty acyl chains<sup>19</sup>, we speculated that  $\omega$ -3 epoxide-containing phospholipids in mast cell membranes might serve as substrates for PAF-AH2 to give rise to free  $\omega$ -3 epoxides. Indeed, LC-MS/MS analysis coupled with multiple-reaction monitoring (MRM) showed the presence of 17,18-EpETE- and 19,20-EpDPE-containing phospholipids in BMDC membrane (Supplementary Fig. 5a–f). The amounts of 17,18-EpETE and 19,20-EpDPE in phospholipids from  $1 \times 10^7$  BMDCs were  $\sim$ 50 ng and  $\sim$ 200 ng, respectively. As wild-type BMDCs released 34 nM ( $\sim$ 100 ng/ $10^7$  cells) and 41 nM ( $\sim$ 150 ng/ $10^7$  cells) of 17,18-EpETE and 19,20-EpDPE, respectively (Fig. 1c), the steady-state levels of  $\omega$ -3 epoxide-containing phospholipids in BMDC membrane seem to be comparable to those of the released  $\omega$ -3 epoxides. When 17,18-EpETE-containing phospholipid membrane was incubated with PAF-AH2, free 17,18-EpETE was produced in a time-dependent manner, with a concomitant reduction in 17,18-EpETE-esterified phospholipids, whereas the PAF-AH2 Ser234Cys mutant failed to release 17,18-EpETE (Fig. 4h,i). Phospholipid species containing non-oxidized EPA or DHA were not hydrolyzed by

PAF-AH2 (Supplementary Fig. 5g). Thus, PAF-AH2 preferentially hydrolyzed  $\omega$ -3 epoxide-containing phospholipids in mast cell membranes to liberate  $\omega$ -3 epoxides.

#### A PAF-AH2 inhibitor suppresses antigen-induced activation of mouse and human mast cells

AA39-2 is a selective inhibitor of PAF-AH2 among the mammalian serine hydrolases<sup>20</sup>. The half-maximal inhibitory concentration (IC<sub>50</sub>) is  $\sim$ 0.17 nM, which is five orders of magnitude lower than the half-maximal cytotoxic concentration ( $\sim$ 35  $\mu$ M). AA39-2, at 1  $\mu$ M, effectively inhibited the PAF-AH2-catalyzed release of 17,18-EpETE from esterified phospholipid precursors in BMDC membranes (Fig. 5a). Antigen-induced degranulation was also reduced by the pretreatment of wild-type BMDCs with AA39-2 (Fig. 5b). AA39-2 did not further reduce the impaired degranulation in *Pafah2*<sup>-/-</sup> BMDCs, supporting the idea that AA39-2 indeed exerts its effect through the inhibition of PAF-AH2. As expected, AA39-2 suppressed antigen-induced phosphorylation of Fc $\epsilon$ RI, Syk and Gab2 in wild-type BMDCs (Fig. 5c,d). In contrast, the degranulation elicited by the calcium ionophore A23187, an IgE-antigen-independent mast cell activator, was not inhibited by AA39-2 (Fig. 5b).

Injection of AA39-2 into the ears attenuated antigen-induced anaphylaxis in wild-type mice but did not further diminish the impaired anaphylaxis in *Pafah2*<sup>-/-</sup> mice (Fig. 5e,f). Moreover, intraperitoneal administration of AA39-2 (1.32  $\mu$ mol per kg body weight, a dose determined by pharmacokinetic studies) suppressed systemic anaphylaxis (Fig. 5g), without obvious signs of hepatic or renal

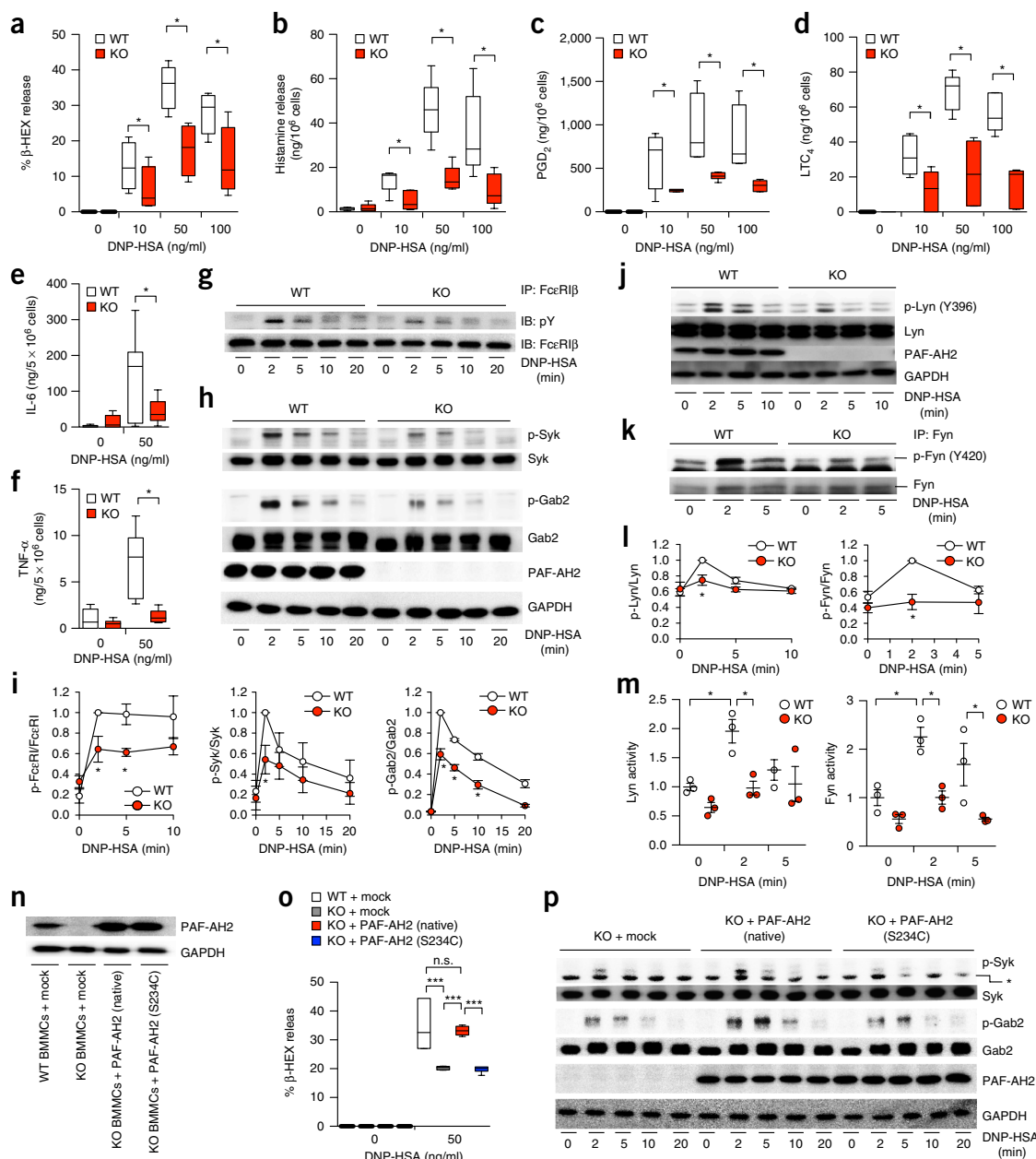


**Figure 3** PAF-AH2 facilitates FcεRI-dependent activation of BMMCs. (**a–f**) Percentage release of β-HEX (degranulation) ( $n = 8$ ) (**a**), release of histamine ( $n = 5$ ) (**b**), production of PGD<sub>2</sub> ( $n = 6$ ) (**c**) and LTC<sub>4</sub> ( $n = 6$ ) (**d**), and secretion of IL-6 ( $n = 10$ ) (**e**) and TNF-α ( $n = 6$ ) (**f**) by wild-type (WT) and *Pafah2*<sup>−/−</sup> (KO) BMMCs. Histamine, PGD<sub>2</sub>, LTC<sub>4</sub>, IL-6 and TNF-α were quantified by ELISA. BMMCs sensitized with anti-DNP IgE were challenged for 30 min (**a–d**) or 6 h (**e,f**) with the indicated concentrations of DNP-HSA as an antigen. (**g–l**) IgE-sensitized wild-type and *Pafah2*<sup>−/−</sup> BMMCs were stimulated with 50 ng/ml DNP-HSA for the indicated times. (**g,k**) Immunoprecipitates of FcεRIβ (**g**) or Fyn (**k**) from BMMC cell lysates were immunoblotted with antibodies to FcεRIβ and phosphotyrosine (pY) (**g**) or with antibodies to phosphorylated Src (p-Src; Tyr416) and Fyn (**k**). (**h,j**) Whole-cell lysates of the BMMCs in **g** were immunoblotted with antibodies to Syk, phosphorylated Syk (p-Syk), Gab2, phosphorylated Gab2 (p-Gab2), Lyn, phosphorylated Lyn (p-Lyn; Tyr396), PAF-AH2 and GAPDH. Blots are representative of three separate experiments with three individual BMMC cultures. (**i,l**) Fold changes in phosphorylated proteins normalized to the corresponding total proteins in given lanes in **g**, **h**, **j** and **k** were determined by densitometric analysis, with the value of wild-type BMMCs stimulated for 2 min set as 1 ( $n = 3$  individual BMMC cultures). (**m**) Whole-cell lysates from the BMMCs in **g** were immunoprecipitated with antibodies to Lyn and Fyn. Kinase activities of the immunoprecipitates were determined by *in vitro* kinase assays. Fold changes in kinase activity were determined relative to the standard kinase activity of c-Src, with the value of unstimulated wild-type BMMCs set as 1 ( $n = 3$  cultures from 3 mice). (**n–p**) Native PAF-AH2 and its catalytically inactive mutant Ser234Cys were retrovirally expressed in *Pafah2*<sup>−/−</sup> BMMCs. (**n**) Immunoblotting of PAF-AH2, with GAPDH as a control. (**o**) Percentage degranulation of antigen-stimulated *Pafah2*<sup>−/−</sup> BMMCs infected with retrovirus bearing an empty vector (mock) or retrovirus with native or mutant PAF-AH2 ( $n = 6$  cultures from 6 mice). (**p**) Phosphorylation of Syk and Gab2 after antigen stimulation as evaluated by immunoblotting. An asterisk indicates a non-specific band. In box plots (**a–f**), center lines indicate the median, box limits represent the upper and lower quartiles, and whiskers extend to the minimum and maximum values. In dot plots and line graphs (**i,l,m**), values are shown as means ± s.e.m. Data were analyzed by one-way ANOVA with Tukey's *post hoc* test (**a–f,m,o**) or by unpaired two-tailed Student's *t* test (**i,l**): \* $P < 0.05$ ; \*\*\* $P < 0.001$ ; n.s., not significant ( $P > 0.05$ ). Data were from one set of experiments (**e,f,m**) or were compiled from two (**b**) or three (**a,c,d,i,l,o**) sets of experiments. Images in **g**, **h**, **j**, **k**, **n** and **p** are representative of three experiments.

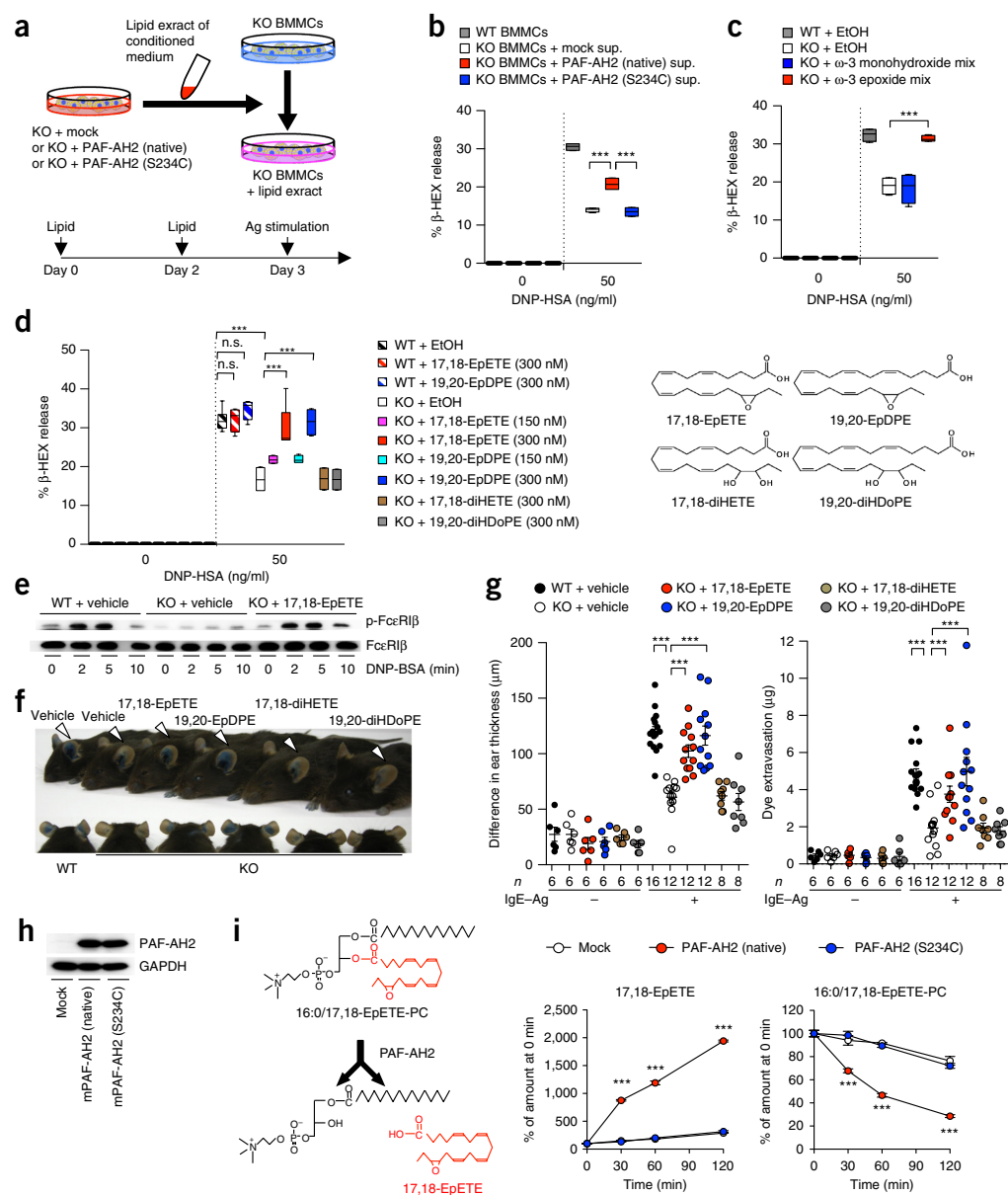


**Figure 4** ω-3 epoxides restore antigen-induced activation of *Pafah2*<sup>-/-</sup> mast cells *in vitro* and *in vivo*. (a) Schematic of the protocol for supplementation of BMMCs with exogenous lipids in culture. KO, *Pafah2*<sup>-/-</sup>. (b) Effects of free fatty acid fractions from *Pafah2*<sup>-/-</sup> BMMCs transfected with an empty vector (mock) or with vector encoding native or mutant (Ser234Cys) PAF-AH2 on IgE-antigen-induced β-HEX release (*n* = 4 cultures from 4 mice). (c,d) Antigen-induced β-HEX release by *Pafah2*<sup>-/-</sup> BMMCs pretreated for 3 d with a mixture (300 nM) of ω-3 monohydroxides (18-HEPE:19-HEPE:20-HEPE:20-HDoHE:21-HDoHE:22-HDoHE at a 1:2:2:1:2:2 ratio) or ω-3 epoxides (17,18-EpETE:19,20-EpDPE at a 2:3 ratio) (c) or with individual lipids (d) in comparison to wild-type BMMCs (*n* = 4 cultures from 4 mice). EtOH, ethanol. (e) Wild-type and *Pafah2*<sup>-/-</sup> BMMCs pretreated with 300 nM 17,18-EpETE or vehicle (EtOH) were stimulated with DNP-HSA for the indicated times. Immunoprecipitates of FcεRIβ were immunoblotted with antibodies to total FcεRIβ and phosphotyrosine. (f,g) Antigen-induced PCA in wild-type and *Pafah2*<sup>-/-</sup> mice after pretreatment for 3 d with the indicated lipids (16 pmol) or vehicle (saline + EtOH). (f) A representative photo is shown. (g) Extravasation of Evans blue in the ears and differences in ear thickness were evaluated. *n* represents the number of mice. (h) Cytosolic fractions of mock-transfected HEK293T cells and HEK293T cells transiently transfected to express native or Ser234Cys mouse PAF-AH2 were subjected to immunoblotting for PAF-AH2 and GAPDH. (i) Hydrolysis of ω-3 epoxide-esterified phospholipids in BMMC membranes by native PAF-AH2 or its mutant. Fold changes in the lipids normalized to corresponding total levels at given time points were determined by the relative ratio of signal intensity, with the value at 0 min set as 100% (*n* = 3 experimental replicates). Values are shown as means ± s.e.m. \*\*\**P* < 0.001, compared with mock at the indicated time points determined by unpaired two-tailed Student's *t* test. Experiments were repeated at least twice, and the data were pooled. In box plots (b–d), center lines indicate the median, box limits represent the upper and lower quartiles, and whiskers extend to the minimum and maximum values. In dot plots (g), values are shown as means ± s.e.m. Data were analyzed by one-way ANOVA with Tukey's *post hoc* test (b–d,g): \**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001; n.s., not significant (\**P* > 0.05). Data were compiled from two (b–d,g) or three (i) sets of experiments. Images in e, f and h are representative of two experiments.

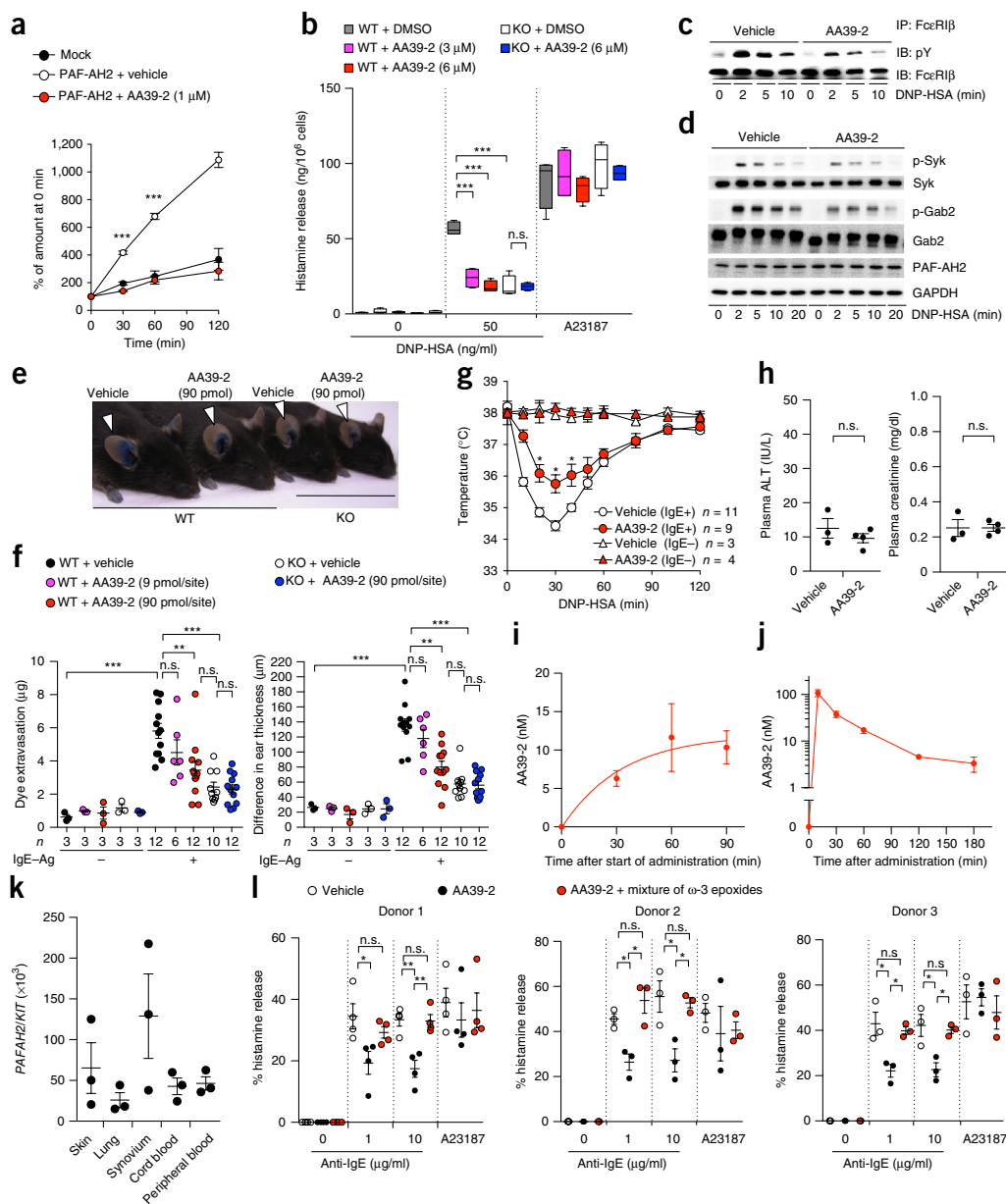


**Figure 5** A PAF-AH2 inhibitor suppresses antigen-induced activation of mouse and human mast cells. **(a)** Effect of AA39-2 on PAF-AH2 activity. The amounts of 17,18-EpETE liberated were monitored as in **Figure 4i** (arbitrarily set to 100% at 0 min;  $n = 3$  experimental replicates). \*\*\* $P < 0.001$ , as compared with mock at the indicated times determined by unpaired two-tailed Student's  $t$  test. **(b)** IgE-antigen- or A23187-dependent histamine release by wild-type (WT) and *Pafah2*<sup>-/-</sup> (KO) BMMCs pretreated for 3 d with vehicle (DMSO) or AA39-2 was quantified by ELISA ( $n = 4$  cultures from 4 mice). **(c,d)** Wild-type BMMCs pretreated with vehicle (DMSO) or 3  $\mu$ M AA39-2 were stimulated with 50 ng/ml DNP-HSA as an antigen for the indicated times. Immunoprecipitates of Fc $\epsilon$ RI $\beta$  were immunoblotted with antibodies to Fc $\epsilon$ RI $\beta$  and phosphotyrosine **(c)** or whole-cell lysates were immunoblotted with antibodies to Syk, p-Syk, Gab2, p-Gab2, PAF-AH2 and GAPDH **(d)**. **(e,f)** Antigen-induced PCA in wild-type and *Pafah2*<sup>-/-</sup> mice after pretreatment with vehicle (saline + DMSO) or AA39-2. **(e)** A representative photo is shown. **(f)** Extravasation of Evans blue in the ears and differences in ear thickness were examined. Experiments were repeated at least twice, and the data were pooled.  $n$  represents the number of mice. **(g)** Rectal temperatures in antigen-induced PCA in wild-type mice treated with vehicle (saline + DMSO) or AA39-2 (1.32  $\mu$ mol per kg body weight intraperitoneally, 1 and 3 d before antigen challenge) after challenge with 500  $\mu$ g of antigen.  $n$  represents the number of mice. \* $P < 0.05$ , vehicle versus AA39-2 after antigen challenge for the indicated periods determined by unpaired two-tailed Student's  $t$  test. **(h)** Plasma ALT and creatinine levels in mice treated with vehicle (saline + DMSO;  $n = 3$  mice) or AA39-2 (1.32  $\mu$ mol per kg body weight intraperitoneally, 1 and 3 d before blood collection) ( $n = 4$  mice). n.s., not significant (\* $P > 0.05$ ) determined by unpaired two-tailed Student's  $t$  test. **(i,j)** Monitoring of plasma AA39-2 levels using LC-MS/MS after continuous intravenous infusion (1.1 nmol/min per kg body weight;  $n = 3$  mice) **(i)** or after intraperitoneal administration (1.32  $\mu$ mol per kg body weight;  $n = 3$  mice) **(j)**. **(k)** Real-time PCR of *PAFAH2* in human mast cells from different sources ( $n = 3$  mice). **(l)** Histamine release from human mast cells treated with AA39-2 in the presence or absence of  $\omega$ -3 epoxides after stimulation with the indicated concentrations of antibody against IgE or 1  $\mu$ M A23187 for 30 min was quantified by ELISA ( $n = 3$  replicate cultures). In box plots **(b)**, center lines indicate the median, box limits represent the upper and lower quartiles, and whiskers extend to the minimum and maximum values. In dot plots and line graph **(a,f,l)**, values are shown as means  $\pm$  s.e.m. Data were analyzed by one-way ANOVA with Tukey's *post hoc* test **(b,f,l)**: \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; n.s., not significant (\* $P > 0.05$ ). Data were from one set of experiments **(a,g-l)** or were compiled from two sets of experiments **(b,f)**. Images in **c-e** are representative of two experiments.

toxicity as monitored by plasma alanine aminotransferase (ALT) and creatinine levels, respectively (Fig. 5h). Thus, pharmacological inhibition of PAF-AH2 suppressed antigen-induced mast cell activation both *in vitro* and *in vivo*, mimicking its genetic deletion.

Pharmacokinetically, the plasma AA39-2 level reached a plateau (11 nM) 60–90 min after its intravenous infusion at a rate of 1.1 nmol/min per kg body weight (Fig. 5i). After a single intraperitoneal injection of AA39-2 (1.32  $\mu$ mol per kg body weight), its plasma level rapidly increased to

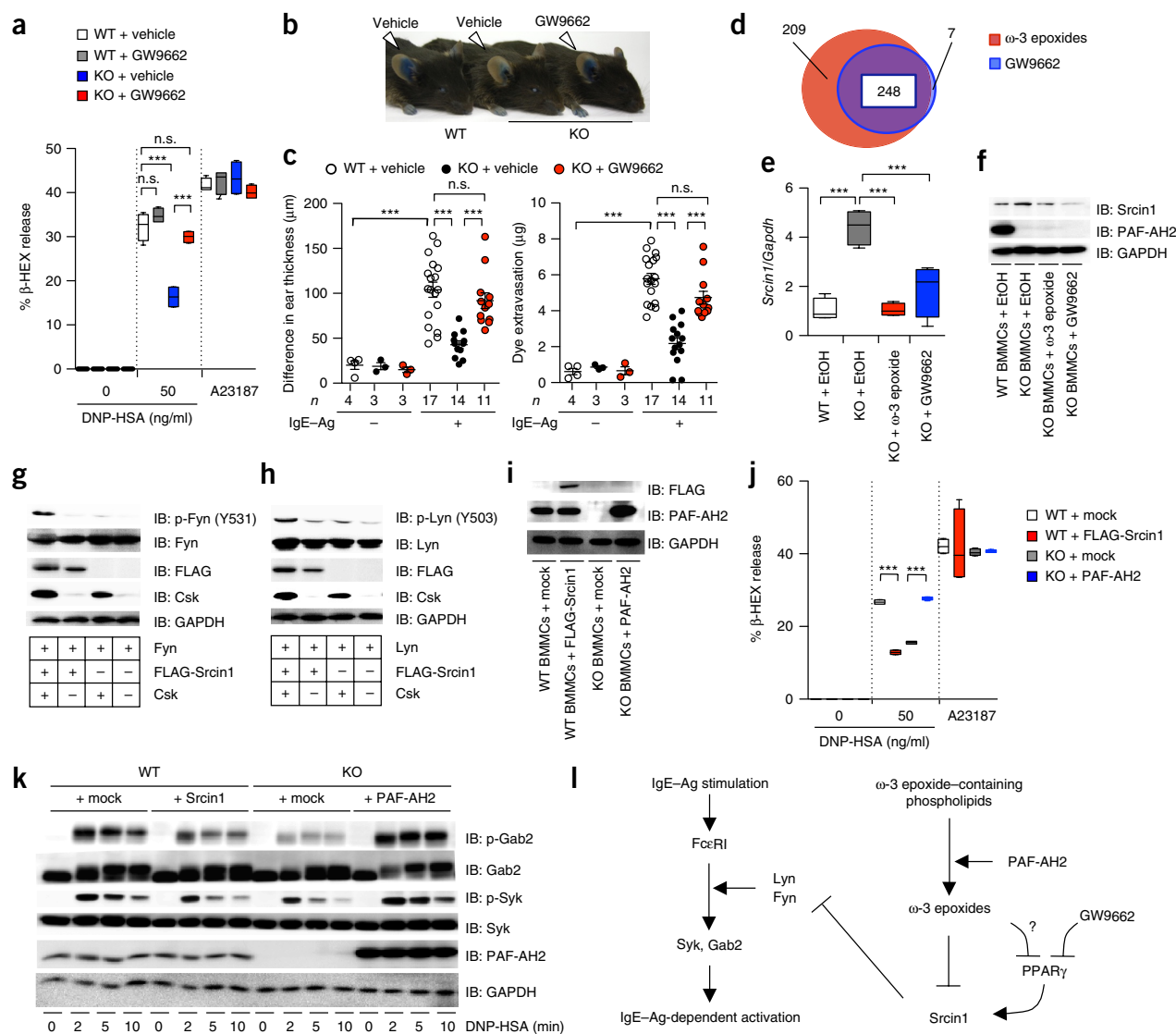


**Figure 6** PAF-AH2-driven  $\omega$ -3 epoxides downregulates Srcin1 expression via inhibiting PPAR $\gamma$ . (a) IgE-antigen- or A23187-dependent  $\beta$ -HEX release by wild-type (WT) and *Pafah2*<sup>-/-</sup> (KO) BMMCs pretreated for 3 d with vehicle (DMSO) or 0.5  $\mu$ M GW9662 ( $n = 4$  cultures from 4 mice). (b,c) Antigen-induced PCA in wild-type and *Pafah2*<sup>-/-</sup> mice after pretreatment for 3 d with vehicle (saline + DMSO) or GW9662 (30 pmol). (b) A representative photo of mice. (c) Extravasation of Evans blue in the ears and differences in ear thickness were measured.  $n$  represents the number of mice. (d) Venn diagram of overlap between downregulated genes in *Pafah2*<sup>-/-</sup> BMMCs treated with a mixture of  $\omega$ -3 epoxides (17,18-EpETE:19,20-EpDPE at a 2:3 ratio; 300 nM) and GW9662 (0.5  $\mu$ M) for 12 h in comparison with *Pafah2*<sup>-/-</sup> BMMCs treated with vehicle. Genes whose expression was suppressed to less than half of that observed in *Pafah2*<sup>-/-</sup> BMMCs treated with vehicle are shown. Each sample corresponded to data pooled from three independent lots of BMMCs. (e) Real-time PCR of *Srcin1* in wild-type and *Pafah2*<sup>-/-</sup> BMMCs pretreated with EtOH, 300 nM 17,18-EpETE or 0.5  $\mu$ M GW9662 for 12 h ( $n = 4$  cultures from 4 mice). (f) Whole-cell lysates from the BMMCs in e were subjected to immunoblotting. (g,h) Lysates from HEK293T cells cotransfected with vectors expressing FLAG-tagged Srcin1, Csk, and either Fyn (g) or Lyn (h) were subjected to immunoblotting. (i) Immunoblotting of FLAG-tagged Srcin1 in wild-type BMMCs. FLAG-tagged Srcin1 or PAF-AH2 was retrovirally expressed in BMMCs, with GAPDH used as a loading control. (j) IgE-antigen- or A23187-induced  $\beta$ -HEX release from wild-type or *Pafah2*<sup>-/-</sup> BMMCs transduced to express FLAG-tagged Srcin1 or PAF-AH2 ( $n = 4$  cultures from 4 mice). (k) Immunoblotting of phosphorylated Syk and Gab2 in wild-type or *Pafah2*<sup>-/-</sup> BMMCs transduced to express FLAG-tagged Srcin1 or PAF-AH2 after antigen stimulation. (l) A schematic diagram of the regulation of Fc $\epsilon$ R1-dependent mast cell activation by PAF-AH2-driven  $\omega$ -3 epoxides. In box plots (a,e,j), center lines indicate the median, box limits represent the upper and lower quartiles, and whiskers extend to the minimum and maximum values. In dot plots (c), values are shown as means  $\pm$  s.e.m. Data were analyzed by one-way ANOVA with Tukey's *post hoc* test (a,c,e,j): \*\*\* $P < 0.001$ ; n.s., not significant (\* $P > 0.05$ ). Data were from one set of experiments (c,d) or were compiled from two sets of experiments (a,e,j). Images in b, f–i and k are representative of two experiments.

>100 nM within 30 min and then declined to 3.3 nM, a concentration that is 19-fold higher than the  $IC_{50}$  (0.17 nM) for PAF-AH2 (ref. 20), and remained nearly constant until ~180 min after injection (Fig. 5j).

High levels of *PAFAH2* expression were also detected in human mast cells prepared from skin, lung, synovium, cord blood and peripheral blood (Fig. 5k). Treatment of human synovial mast cells from three donors with AA39-2 substantially inhibited histamine release induced by FcεRI cross-linking with antibody against IgE. The impaired histamine release was fully reversed by addition of a mixture of 17,18-EpETE and 19,20-EpDPE (Fig. 5l). Thus, the PAF-AH2-driven epoxides also contributed to the activation of human mast cells.

### ω-3 epoxides act through a pathway involving PPARγ

The impaired response of *Pafah2*<sup>-/-</sup> BMMCs to antigen stimulation was almost fully restored by pretreatment with ω-3 epoxides for 3 d (Fig. 4d), whereas a shorter pretreatment (4 h) had little effect (Supplementary Fig. 6a). We therefore hypothesized that gene transcription might be one of the mechanisms underlying the observed effect of ω-3 epoxides. Because the nuclear transcription factors PPARγ and PPARδ, which bind to fatty acids or their derivatives as ligands, are expressed in mouse BMMCs<sup>7</sup>, we examined the effects of their known agonists and antagonists on antigen-induced activation of *Pafah2*<sup>-/-</sup> BMMCs. Among the agents tested, the PPARγ antagonist GW9662, like ω-3 epoxides (Fig. 4d), fully restored degranulation in *Pafah2*<sup>-/-</sup> BMMCs, without affecting degranulation in wild-type BMMCs (Fig. 6a and Supplementary Fig. 6b). GW9662 also restored antigen-mediated PCA in *Pafah2*<sup>-/-</sup> mice (Fig. 6b,c). In microarray profiling of ~30,000 genes in *Pafah2*<sup>-/-</sup> BMMCs, GW9662 and ω-3 epoxides downregulated 457 genes and 255 genes, respectively, by a factor of at least two. Intriguingly, the two groups shared 248 genes (Fig. 6d and Supplementary Table 1). These results suggest that the PPARγ antagonist mimics the action of ω-3 epoxides, supporting the idea that the ω-3 epoxide-driven effects are mediated largely, if not solely, through the inhibition of PPARγ (Fig. 6l). However, in an *in vitro* reporter gene assay where rosiglitazone or 15-deoxy-Δ<sup>12,14</sup>-PGJ<sub>2</sub> was used as a PPARγ agonist, neither 17,18-EpETE nor 19,20-EpDPE suppressed the agonist-induced PPARγ activation (Supplementary Fig. 6c), suggesting that ω-3 epoxides functionally interact with PPARγ in one or more indirect ways.

### ω-3 epoxides regulate mast cell activation by reducing Srcin1 expression

To gain further insight into the action of ω-3 epoxides, we tried to identify gene products that were similarly downregulated by ω-3 epoxides and GW9662 and could suppress FcεRI-proximal signaling. Because antigen-induced phosphorylation of FcεRI, Syk and Gab2 was suppressed in *Pafah2*<sup>-/-</sup> BMMCs and restored by ω-3 epoxides (Fig. 4e and Supplementary Fig. 4d), we assumed that a PAF-AH2-ω-3 epoxide axis would regulate the expression of certain genes that could affect the expression or activity of the FcεRI-proximal Src-family kinases Lyn and Fyn<sup>2</sup>. Indeed, Src-kinase-signaling inhibitor 1 (*Srcin1*)<sup>21</sup> was one of the genes commonly downregulated by ω-3 epoxides and GW9662 (Supplementary Table 1). qRT-PCR and western blotting confirmed that the expression levels of *Srcin1* mRNA and protein, respectively, were higher in *Pafah2*<sup>-/-</sup> BMMCs than in wild-type BMMCs, and the levels in *Pafah2*<sup>-/-</sup> BMMCs were downregulated to those in wild-type BMMCs by addition of ω-3 epoxides or GW9662 (Fig. 6e,f). *Srcin1* binds directly and simultaneously to Src and another protein tyrosine kinase, Csk, leading to Csk-dependent phosphorylation of an

inhibitory tyrosine residue in the C-terminal region of Src and thus to Src inactivation<sup>21</sup>. Because it remained unclear whether *Srcin1* could also inactivate other Src-family kinases, including Fyn and Lyn, we examined the phosphorylation states of inhibitory tyrosine residues in the C-terminal regions of Fyn (Tyr531) and Lyn (Tyr503)<sup>22</sup> in HEK293T cells cotransfected with Csk and FLAG-tagged *Srcin1*. Indeed, the inhibitory residues of Fyn (Fig. 6g) and Lyn (Fig. 6h) were phosphorylated only in the presence of both *Srcin1* and Csk. In addition, *Srcin1* was coimmunoprecipitated with Fyn or Lyn only in the presence of Csk (Supplementary Fig. 6d,e).

When FLAG-tagged *Srcin1* was retrovirally expressed in wild-type BMMCs, in which the expression level of endogenous *Srcin1* is lower than in *Pafah2*<sup>-/-</sup> BMMCs (Fig. 6e), antigen-induced degranulation and phosphorylation of Syk and Gab2 were reduced (Fig. 6i-k). Conversely, knockdown of *Srcin1* in *Pafah2*<sup>-/-</sup> BMMCs by *Srcin1*-targeted shRNA restored antigen-induced degranulation and phosphorylation of Syk and Gab2 (Supplementary Fig. 6f-h). Other genes commonly altered by ω-3 epoxides and GW9662 included dual-specificity phosphatase 22 (*Dusp22*), which inhibits the kinase activity of another Src-family kinase, Lck<sup>23</sup>, and phosphatidylinositol 3-kinase (PI3K) catalytic subunit type 2α (*Pik3c2a*), which controls PI3K signaling<sup>24</sup> (Supplementary Table 1). *Dusp22* was also downregulated by ω-3 epoxides in *Pafah2*<sup>-/-</sup> BMMCs; however, unlike the case for *Srcin1*, its knockdown did not restore degranulation of *Pafah2*<sup>-/-</sup> BMMCs (Supplementary Fig. 6i,j). Because *Pik3c2a* was upregulated by ω-3 epoxides in *Pafah2*<sup>-/-</sup> BMMCs, we hypothesized that its knockdown in wild-type BMMCs might result in a phenotype mimicking the impaired activation of *Pafah2*<sup>-/-</sup> BMMCs. However, wild-type BMMCs with *Pik3c2a* knockdown did not phenocopy *Pafah2*<sup>-/-</sup> BMMCs. Taken together, these results suggest that *Srcin1* blocks phosphorylation of FcεRI by inhibiting Lyn and Fyn and that PAF-AH2-driven ω-3 epoxides downregulate *Srcin1* expression by counteracting PPARγ signaling to ensure full activation of mast cells.

## DISCUSSION

In the present study, we have demonstrated that ω-3 epoxides including 17,18-EpETE and 19,20-EpDPE, which are stored as esterified forms in membrane phospholipids of mast cells, are constitutively released by PAF-AH2, an oxidized-phospholipid-selective PLA2. These ω-3 epoxides suppress the transcription of *Srcin1*, which prevents activation of the FcεRI-proximal kinases Lyn and Fyn. Thus, the PAF-AH2-ω-3 epoxides-*Srcin1* axis represents a new signaling module that ensures full IgE-antigen-induced activation of mast cells. A PPARγ antagonist mimics the action of ω-3 epoxides, implying that ω-3 epoxides regulate *Srcin1* through a pathway involving PPARγ (Fig. 6l).

Among a wide variety of bioactive substances that modulate immunological responses, lipid mediators represent a class of critical regulators of the functions of mast cells. Accordingly, much attention has been paid to AA-derived eicosanoids that are produced upon stimulation of mast cells or supplied spatiotemporally from neighboring cells to mast cells. These mediators are mostly produced via the conventional eicosanoid-biosynthetic pathways controlled by cPLA2α (ref. 6). PLA2G3, another PLA2 secreted from mast cells, acts on neighboring fibroblasts to produce a distinct pool of PGD<sub>2</sub>, which in turn promotes mast cell maturation<sup>25</sup>. PAF, another proinflammatory lipid mediator produced by activated mast cells and leukocytes, is degraded mostly by lipoprotein-associated PLA2 (plasma-type PAF-AH) in circulation, which may help to limit the severity of anaphylaxis<sup>26</sup>. In the present study, we identified a new PLA2-driven lipid mediator

pathway in mast cells. Unstimulated mast cells spontaneously produce various EPA and DHA metabolites, including  $\omega$ -3 monohydroxides and epoxides, which are generated by PAF-AH2, a unique PLA2 that selectively releases oxidized fatty acids from phospholipids. Concurrently,  $\omega$ -3 epoxides exhibit vasodilatory and cardioprotective effects<sup>27</sup>, ameliorate obesity-associated metabolic disorders<sup>28</sup>, and prevent pathogenic angiogenesis and tumor progression<sup>29</sup>. The present results reveal a previously unrecognized role for  $\omega$ -3 epoxides, 17,18-EpETE and 19,20-EpDPE, in allergy.

The regulatory actions of cPLA2 $\alpha$  and PAF-AH2, which are two major intracellular PLA2 enzymes expressed in mast cells, are distinct. In the conventional biosynthetic pathway, Fc $\epsilon$ RI cross-linking followed by calcium influx triggers the stimulus-coupled, cPLA2 $\alpha$ -driven release of AA, which is converted to eicosanoids<sup>8–10</sup>. In contrast, 17,18-EpETE and 19,20-EpDPE are released from their esterified precursors, that is,  $\omega$ -3 epoxide-containing phospholipids, by PAF-AH2 under resting conditions. cPLA2 $\alpha$ -driven  $\omega$ -6 eicosanoids and PAF-AH2-driven  $\omega$ -3 epoxides may coexist in the tissue microenvironments where mast cells are exposed to antigen. Because genetic depletion of cPLA2 $\alpha$  does not impair mast cell degranulation and PCA reaction<sup>25</sup>, it is unlikely that the  $\omega$ -6 and  $\omega$ -3 lipid mediators driven by the two distinct PLA2 enzymes work cooperatively in the process of mast cell activation. It remains unknown (i) whether  $\omega$ -3 fatty acids esterified into phospholipids are directly oxygenated and (ii) whether free  $\omega$ -3 fatty acids are first oxygenated and then esterified into phospholipids. In support of the latter idea, we and others have shown that some oxygenated fatty acids are efficiently incorporated into phospholipids in living cells<sup>30</sup>. What is the advantage of having bioactive oxygenated fatty acids in the form of their esterified precursors? Because free fatty acid epoxides are readily hydrolyzed to dihydrodiols by cytosolic sEH, phospholipids may act as stable reservoirs of otherwise fragile  $\omega$ -3 epoxides to ensure their continuous supply within cells. Assuming that prolonged stimulation of nuclear receptors or alternative downstream effectors requires a low but sustained supply of the cognate ligands, continuous release of bioactive  $\omega$ -3 epoxides from their esterified precursors may help to maintain cellular homeostasis. Indeed, 17,18-EpETE and 19,20-EpDPE affect the transcription of a series of genes that largely overlap with those regulated by PPAR $\gamma$ , implying that these  $\omega$ -3 epoxides act by counteracting signaling by this nuclear lipid receptor.

PPAR $\gamma$  negatively regulates antigen-induced mast cell activation<sup>7,31,32</sup>, whereas  $\omega$ -3 epoxides relieve this PPAR $\gamma$ -mediated suppression in mast cells, allowing their optimal activation. Accordingly, genetic or pharmacological inactivation of PAF-AH2, an endogenous supplier of  $\omega$ -3 epoxides, dampens this homeostatic regulation. Our attempt to demonstrate the direct antagonism of PPAR $\gamma$  by  $\omega$ -3 epoxides in a reporter assay was unsuccessful, possibly because the present assay conditions using an artificial reporter gene and PPAR $\gamma$  agonists (rosiglitazone and 15-deoxy- $\Delta^{12,14}$ -PGJ<sub>2</sub>) might be inadequate or because  $\omega$ -3 epoxides might modulate PPAR $\gamma$  activity in an indirect way through altering post-translational modifications (for example, phosphorylation and acetylation) of PPAR $\gamma$ , recruiting co-activators/repressors, interacting with other regulatory factors such as their putative receptor(s) or being converted into further metabolites, one or more of which may serve as bona fide PPAR $\gamma$  antagonists. Of note, pretreatment with  $\omega$ -3 epoxides for >1 d was required to fully restore the impaired response of *Pafah2*<sup>-/-</sup> BMMCs. PPAR $\gamma$  regulates the expression of downstream genes directly by binding to its target gene promoters or indirectly by regulating the expression of other transcription factors or histone lysine

methyltransferases<sup>33,34</sup>. In the latter case, expression of some downstream genes often takes more than 24 h after PPAR $\gamma$  activation<sup>35,36</sup>. We speculate that these PPAR $\gamma$ -associated processes may provide one of the reasons why the restoration effect on *Pafah2*<sup>-/-</sup> BMMCs requires a relatively long time period following exposure to  $\omega$ -3 epoxides. Nonetheless, further studies are needed to fill in the picture of the mechanistic action for this event.

Our results show that, in mast cells, Srcin1 is induced by deprivation of  $\omega$ -3 epoxides and functions as a scaffold that bridges Csk and two Src-family kinases (Lyn and Fyn) to suppress Fc $\epsilon$ RI signaling. This mode of action for Srcin1 is reminiscent of that for Csk-binding protein (Cbp), which also recruits Csk to phosphorylate Src, thereby terminating Fc $\epsilon$ RI signaling<sup>37</sup>. A notable difference between Cbp and Srcin1 is that Cbp is expressed constitutively and activated post-translationally by phosphorylation immediately after Fc $\epsilon$ RI aggregation to avoid harmful mast cell hyperactivation, whereas Srcin1 is induced transcriptionally only when the PAF-AH2-mediated homeostatic supply of endogenous  $\omega$ -3 epoxides is limited.

$\omega$ -3 fatty acids are generally believed to be beneficial to the host. However, the effects of  $\omega$ -3 fatty acids in allergy have been controversial<sup>4,5,38–40</sup>, and immune suppression by  $\omega$ -3 fatty acid metabolites could be disadvantageous in host defense<sup>41–43</sup>. Fc $\epsilon$ RI-dependent mast cell activation originally evolved as a defense system against harmful venom, bacteria and parasites. From this standpoint,  $\omega$ -3 epoxide-mediated optimization of Fc $\epsilon$ RI signaling may serve to maintain a healthy state. In a modern Western lifestyle, however, this protective response has been shifting to deleterious outcomes against excessive exposure to environmental allergens, manifesting as allergic diseases. Therefore, the actions of  $\omega$ -3 fatty acids may depend on the pathophysiological state.

Given that pharmacological inhibition of PAF-AH2 reduces antigen-induced activation of mouse and human mast cells and that *Pafah2*<sup>-/-</sup> mice do not show any other apparent abnormalities, a PAF-AH2-specific inhibitor could be useful as a novel antiallergic drug.

## METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the [online version of the paper](#).

*Note: Any Supplementary Information and Source Data files are available in the online version of the paper.*

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## AUTHOR CONTRIBUTIONS

Y.S. and N.K. designed and performed the experiments, analyzed the data, interpreted the results and wrote the manuscript. Y. Taketomi performed the experiments with mast cell engraftment and interpreted the results. M.A. performed comprehensive lipidomics analysis. Y.O. aided in experiments with

human mast cells. Y. Tanaka performed experiments with electroporation-mediated gene silencing. Y.N. performed microarray analysis. T.M. and H.K. performed pharmacokinetic studies and interpreted the results. A.A. and B.C. supplied reagents and interpreted the results. M.M. and H.A. designed the experiments, interpreted the results and wrote the manuscript.

#### COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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## ONLINE METHODS

**Antibodies.** Antibodies to Syk (sc-1077), Lyn (sc-15), Fyn (sc-16) and Fgr (sc-74542) were from Santa Cruz Biotechnology. Antibodies to phosphorylated Syk (Tyr525/526; 2711), phosphorylated Gab2 (Tyr452; 3881), Gab2 (3239), phosphorylated Lyn (Tyr507; 2731), Csk (4980), phosphorylated Src (Tyr416; 2101) and Srcin1 (3757) were purchased from Cell Signaling Technology. An antibody to Hck (ab75839) was from Abcam. An antibody to GAPDH (6C5) was from Calbiochem. An antibody to phosphotyrosine (4G10) was from Upstate Biotechnology. Antibodies to phosphorylated Fyn (Tyr530; SAB4503872) and PAF-AH2 (HPA018157) were from Sigma-Aldrich. Monoclonal mouse antibody to FcεRIβ (clone JRK) was kindly provided by J. Rivera (National Institutes of Health)<sup>44</sup>.

**Mice.** *Pafah2*<sup>-/-</sup>, *Pla2g4a*<sup>-/-</sup> and *Pnpla8*<sup>-/-</sup> mice have been described previously<sup>45,10,46</sup>. All mice were backcrossed to C57BL/6 mice for 12 generations except for the experiment in **Supplementary Figure 2a–c**. Mice in **Supplementary Figure 2a–c** were backcrossed to the BALB/c background for nine generations. In all experiments using knockout mice (male, 12–14 weeks old), the knockout mice were compared with age-matched littermate control mice or wild-type mice purchased from CLEA Japan. Mast cell-deficient *Kit*-mutant mice (C57BL/6J-*Kit*<sup>W<sup>sh</sup>/W<sup>sh</sup>) were purchased from the Jackson Laboratories. All mice were housed in climate-controlled (23 °C) specific-pathogen-free facilities with a 12-h light/12-h dark cycle, with free access to standard laboratory food (CE2, CLEA) and water. All animal experiments were performed in accordance with protocols approved by the Animal Committee of the University of Tokyo and the Tokyo Metropolitan Institute of Medical Science in accordance with the Standards Relating to the Care and Management of Experimental Animals in Japan.</sup>

**Anaphylaxis.** For antigen-induced PCA, 30 ng of monoclonal anti-DNP IgE (SPE-7, Sigma-Aldrich) was intradermally injected into both ears of mice. After 24 h, the mice were challenged by intravenous injection with 60 μg of DNP-conjugated HSA (Sigma-Aldrich) in saline containing Evans blue dye (Wako). Extravasation of blue dye in the ear was monitored for 30 min, and dissected ears were incubated at 37 °C in 3 N KOH. Then, Evans blue dye was extracted in acetone. Quantitative analysis of the extracts was performed by measuring the absorbance of blue dye at 620 nm. Ear thickness was measured 30 min after antigen challenge using a dial thickness gauge (Mitutoyo Corporation). Differences in ear thickness were determined as the difference in thickness before and after antigen challenge. For compound 48/80-induced anaphylaxis, the ears were intradermally injected with 100 ng of compound 48/80 (Sigma-Aldrich) followed by intravenous injection with 1 mg of Evans blue. After 30 min, Evans blue extravasation was measured as described above. For PSA, mice were administered 3 μg of anti-DNP IgE intravenously. After 24 h, the mice were challenged with 500 μg of DNP-HSA. After antigen challenge, rectal temperature was monitored for 120 min with an electronic thermometer (Physitemp Instruments). As required for experiments, AA39-2 was administered intraperitoneally 1 and 3 d before antigen challenge. Postcaval blood samples of the sacrificed mice were collected 1.5 min after antigen challenge. The sera were subjected to measurement of histamine by ELISA in accordance with the manufacturer's instructions (Immunotech, MBL).

**Histological analysis.** Ears were fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections (5 μm thick) were cut using a microtome and stained with 0.05% toluidine blue (pH 0.5) for the detection of mast cells. Degranulated mast cells were defined as those showing the release of cellular granules. For immunohistochemistry, dorsal skin specimens were frozen in OCT compound (Sakura Finetek) and cut using a cryostat. 10-μm sections were dried overnight at room temperature and incubated overnight at 4 °C with a mouse monoclonal antibody to human PAF-AH2 (TI10), which reacted with PAF-AH2 but not any other proteins on an immunoblot of mouse tissue lysates<sup>45,11</sup>. A method using an avidin–biotin–peroxidase complex (Histofine, Nichirei Biosciences) was then employed followed by counterstaining with toluidine blue.

**Culture of mouse BMMCs.** To prepare BMMCs, bone marrow cells obtained from mice were cultured in IL-3-containing BMMC complete medium. After 4–6 weeks of culture, >95% of the floating cells were confirmed to be Kit<sup>+</sup>FcεRIα<sup>+</sup> mast cells by flow cytometry using a FACSCalibur instrument (Becton Dickinson).

**Activation of BMMCs.** Before stimulation, BMMCs were incubated with 1 μg/ml anti-DNP IgE for 3 h and then washed twice with Tyrode's buffer (20 mM HEPES pH 7.4, 135 mM NaCl, 5 mM KCl, 5.6 mM glucose, 1.8 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub> and 0.5 mg/ml BSA (Sigma-Aldrich)). BMMCs (10<sup>6</sup> cells) were stimulated with various concentrations (typically 50 ng/ml) of DNP-HSA (Sigma-Aldrich), 10 μM A23187 (Sigma-Aldrich) or ATP (Cayman Chemicals). Degranulation was evaluated by the amounts of released β-HEX in an enzymatic assay using 4-nitrophenyl *N*-acetyl-β-glucosaminide or the amounts of histamine as determined by ELISA. The amounts of eicosanoids and cytokines were determined by ELISA kit following the manufacturer's instructions (Cayman Chemical).

**Transmission electron microscopy.** BMMCs were fixed with 0.1 M sodium cacodylate buffer (pH 7.2) containing 1% glutaraldehyde and 4% paraformaldehyde, postfixed with 2% OsO<sub>4</sub> in PBS, dehydrated by a graded ethanol series, passed through propylene oxide and embedded in Poly/Bed812 EPON (Polyscience). Ultrathin sections (0.08 μm thick) were stained with uranyl acetate and lead citrate and then examined using an electron microscope (H-7600, Hitachi).

**Adoptive transfer of BMMCs into mast cell-deficient mice.** BMMCs (10<sup>6</sup> cells) were reconstituted by intradermal injection into 6-week-old male *Kit*<sup>W<sup>sh</sup>/W<sup>sh</sup> mice. Forty days after reconstitution, antigen-induced PCA was induced, as described above. The distribution and maturation of reconstituted BMMCs in tissue were evaluated by toluidine blue staining or by real-time PCR of mast cell marker genes.</sup>

**In vitro kinase assays.** Lyn, Fyn, Fgr and Hck immunoprecipitates from antigen-stimulated BMMCs were assayed for their kinase activities using an ELISA-based Universal Tyrosine Kinase Assay kit (TaKaRa) according to the manufacturer's instructions. The activities were defined as the units of cellular Src kinase activity.

**Preparation of ω-3 epoxide-esterified phospholipids.** To prepare ω-3 epoxide-containing phospholipids, 10<sup>7</sup>*Pafah2*<sup>-/-</sup> BMMCs were incubated for 2 h with 9 μM 17,18-EpETE (Cayman Chemicals) or 19,20-EpDPE (Cayman Chemicals) that had been dissolved in 500 μM Tyrode's buffer (135 mM NaCl, 5 mM KCl, 20 mM HEPES, 5.6 mM glucose, 1 mM MgCl<sub>2</sub>, 1.8 mM CaCl<sub>2</sub> and 0.05% BSA, pH 7.4) containing 5 mg/ml essentially fatty acid-free BSA. After incubation, the suspension was centrifuged at 400g for 10 min and washed once with fresh Tyrode's buffer. Phospholipids were extracted by the method of Bligh and Dyer<sup>47</sup>. To prepare the membrane fraction containing ω-3 epoxide-esterified phospholipids, ω-3 epoxide-treated *Pafah2*<sup>-/-</sup> BMMCs were disrupted by sonication and cell lysates were centrifuged at 100,000g for 1 h at 4 °C to separate the cytosol (supernatant) and membrane (pellet) fractions. The membrane fraction was dissolved in assay buffer containing 50 mM Tris-HCl (pH 7.4), 5 mM EDTA and 2 mM DTT. The suspension was homogenized by 50 strokes of a loose-fitting dounce homogenizer and sonicated.

**Lipid extraction from the conditioned media of BMMCs.** To obtain conditioned media from BMMCs, 2 × 10<sup>7</sup> BMMCs were cultured in IL-3-containing BMMC complete medium for 4 d. Neutral lipids, phospholipids and free fatty acids were extracted from the conditioned media by solid-phase extraction using Sep-Pak C18 cartridges (Waters). Briefly, the conditioned medium was added to Sep-Pak columns and neutral lipids were eluted with 10 ml of hexane. Free fatty acids were subsequently eluted with 10 ml of methyl formate. Phospholipids were finally eluted with 10 ml of methanol.

**Treatment of BMMCs with lipids or AA39-2.** Lipids and AA39-2 (ref. 20) were dissolved in ethanol and DMSO, respectively, and added to BMMCs twice in 3 d. IgE-antigen-induced β-HEX release was evaluated a day after the second treatment with these agents.

**Electrospray ionization mass spectrometry.** For the quantification of fatty acids, LC–MS/MS-based lipidomics analyses were performed using an ultra-high-performance liquid chromatography (UPLC) system (Waters) with a linear ion-trap quadrupole mass spectrometer (QTRAP5500, AB SCIEX) equipped with an Acquity UPLC BEH C18 column (Waters). MS/MS analyses were

conducted in negative-ion mode, and fatty acid metabolites were identified and quantified by MRM.

For the detection of phospholipids, LC–MS/MS-based lipidomics analyses were performed on a Shimadzu Nexera UFLC system (Shimadzu) coupled with a QTRAP 4500 hybrid triple-quadrupole linear ion-trap mass spectrometer (AB SCIEX). Lipids extracted from BMMCs were injected by an autosampler; typically, 10  $\mu$ l (3 nmol phosphorous equivalent) of the sample was applied. Chromatographic separation was performed on an Acquity UPLC HSS T3 column (100 mm  $\times$  2.1 mm, 1.8  $\mu$ m; Waters) maintained at 40 °C using mobile phase A (water:methanol (50:50) containing 10 mM ammonium acetate and 0.2% acetic acid) and mobile phase B (isopropanol:acetone (50:50)) in a gradient program (0–3 min: 30% B  $\rightarrow$  50% B; 3–24 min: 50% B  $\rightarrow$  90% B; 24–28 min: 30% B) with a flow rate of 0.3 ml/min. The instrument parameters for negative-ion mode were as follows: curtain gas, 10 psi; collision gas, 7 arbitrary units; ionspray voltage, –4,500 V; temperature, 700 °C; ion source gas 1, 30 psi; ion source gas 2, 70 psi. The instrument parameters for positive-ion mode were as follows: curtain gas, 10 psi; collision gas, 7 arbitrary units; ionspray voltage, 4,500 V; temperature, 700 °C; ion source gas 1, 30 psi; ion source gas 2, 50 psi. Specific detection was performed by MRM as described in **Supplementary Table 2**.

The characteristic fragmentation patterns of individual phospholipid molecular species were determined by enhanced product ion scanning (EPI). MS/MS analyses were conducted in negative-ion mode. The instrument parameters for negative-ion mode were as follows: curtain gas, 10 psi; collision gas, high; ionspray voltage, –4,500 V; temperature, 700 °C; ion source gas 1, 30 psi; ion source gas 2, 80 psi; declustering potential, –105 V; entrance potential, –10 V; collision energy, –52 V; collision cell exit potential, –20 V. The linear ion-trap fill time was set to “dynamic fill time.” The scan range of the instrument was set at 50–900  $m/z$ , at a scan speed of 10,000 Da/s.

AA39-2 was quantified by the aforementioned LC–MS/MS system (a Shimadzu Nexera UFLC system coupled with a QTRAP5500 and an Acquity UPLC BEH C18 column) using mobile phase A (0.1% formic acid in water) and mobile phase B (acetonitrile) in a gradient program (0–2 min: 10% B  $\rightarrow$  100% B; 2–5 min: 100% B; 5–7 min: 10% B) with a flow rate of 0.3 ml/min. The instrument parameters were as follows: curtain gas, 10 psi; collision gas, 8 arbitrary units; ionspray voltage, 5,000 V; temperature, 700 °C; ion source gas 1, 80 psi; ion source gas 2, 40 psi; declustering potential, 66 V; entrance potential, 10 V; collision energy, 15 V; collision cell exit potential, 12 V. The amount of AA39-2 was quantified by MRM using the transition 319  $\rightarrow$  98  $m/z$  based on a calibration curve constructed from the analysis of 0.24 to 240 nM authentic standard solutions prepared using blank plasma.

**Chemical hydrolysis of lipids.** Lipids extracted from BMMCs were fractionated into phospholipids, free fatty acids and neutral lipids by solid-phase extraction using Sep-Pak C18 cartridges as described above. The purified lipid fractions were dried under nitrogen gas. The resulting residues were dissolved in 1 ml of 0.2 N KOH in methanol (1:5) and incubated for 60 min at 50 °C. After incubation, an aliquot of HCl was added to adjust the pH of the lipid mixture to 4.0. The free fatty acid fractions were obtained using Sep-Pak C18 cartridges, and quantification of specific fatty acids was performed by MRM as described in **Supplementary Table 2**.

**Biochemical assays.** Enzymatic hydrolysis of  $\omega$ -3 epoxide-containing phospholipids was measured as follows. The standard incubation system for the assay comprised 50 mM Tris-HCl (pH 7.4), 5 mM EDTA, 2 mM DTT, the membrane fraction containing  $\omega$ -3 epoxide-esterified phospholipids, and the cytosol fraction obtained from HEK293T cells transfected with empty vector, native PAF-AH2 or Ser234Cys mutant with or without 1  $\mu$ M AA39-2 in a total volume of 125  $\mu$ l. After incubation for appropriate periods of time at 37 °C, the reactions were stopped by adding 1.25 ml of chloroform:methanol (4:1), followed by addition of 125  $\mu$ l of water and 16.6 pmol of 1-dodecanoyl-2-tridecanoyl PC as an internal standard. The lipids in the organic phase were extracted and reconstituted in isopropanol:methanol (1:1). The lipid extracts were separated by UPLC, and 17,18-EpETE and 16:0/17,18-EpETE-PC were quantified using MRM, as described above.

Plasma creatinine levels were measured using LabAssay Creatinine (Wako). Plasma ALT levels were measured using the Transaminase CII Testwako kit (Wako).

**Pharmacokinetic studies.** In the intravenous infusion studies, AA39-2 (1.1 nmol/min per kg body weight) was infused into C57BL/6 mice (male, 10 weeks old) via the jugular vein under anesthesia with isoflurane. Blood samples were collected via the jugular vein at 30, 60 and 90 min after the start of the infusion and immediately centrifuged at 20,000g for 5 min to obtain the plasma fraction. The plasma fraction was mixed with three volumes of acetonitrile and centrifuged at 15,000 r.p.m. for 5 min. The supernatants were filtered and subjected to LC–MS/MS as described above. All tubes and syringes for plasma collection were pretreated with 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF; Wako), a serine esterase inhibitor, to prevent possible hydrolysis of AA39-2 after blood collection. In the intraperitoneal administration studies, AA39-2 (1.32  $\mu$ mol per kg body weight) was given to C57BL/6 mice (male, 10 weeks old) intraperitoneally. AA39-2 in blood samples was analyzed as above.

**Studies using human mast cells.** Preparation and culture of mast cells from human synovium, cord blood, peripheral blood, skin and lung were performed as described previously<sup>48,49</sup>. Human tissue was obtained during surgery at the Nihon University Hospital under approval of the Ethics Committees of the Nihon University School of Medicine, and all subjects provided written informed consent in accordance with the Declaration of Helsinki of the World Medical Association. Synovial tissues were obtained from patients with osteoarthritis who were undergoing joint replacement surgery. Skin tissues, cord blood and peripheral blood were obtained from healthy donors. Macroscopically normal lung tissues were obtained from patients with lung cancer. The purity of human mast cells, as assessed with metachromatic staining, was more than 97%. Cultured human synovium mast cells were pretreated with AA39-2 (3  $\mu$ M) and/or a mixture of  $\omega$ -3 epoxides (300 nM) twice in 3 d or were left untreated and were then sensitized with 1 mg/ml recombinant human myeloma IgE (Calbiochem) at 37 °C for 24 h. After washing, the cells were challenged with rabbit antibody against human IgE (Dako) or 1  $\mu$ M A23187 (Sigma-Aldrich) at 37 °C for 30 min. After incubation, cell-free culture supernatants and cell pellets were harvested to quantify histamine levels.

**Microarrays.** Total RNA was extracted from BMMCs derived from *Pafah2*<sup>–/–</sup> mice treated with a mixture of  $\omega$ -3 epoxides or vehicle and purified using the RNeasy Mini kit (Qiagen). The quality of the RNA was validated with a 2100 Bioanalyzer (Agilent Technologies). Purified RNA was amplified and labeled using the Low-Input Quick Amp Labeling kit according to the manufacturer’s protocol (Agilent Technologies). Cy3-labeled cRNA was fragmented using the Agilent Gene Expression Hybridization kit. Samples were hybridized to the Whole Mouse Genome DNA Microarray (4  $\times$  44K; Agilent Technologies). The slides were scanned using a SureScan Microarray Scanner (Agilent Technologies). Microarray data were analyzed with Feature Extraction software (Agilent Technologies) and then normalized by a 75-percentile shift using GeneSpring GX software (Agilent Technologies).

**Statistical analysis.** Data were converted to means  $\pm$  s.e.m., and the unpaired Student’s test was applied to determine significant differences between two samples. Statistical differences between multiple treatment groups and a control group were determined using ANOVA and the Tukey–Kramer *post hoc* test. Sample sizes were chosen on the basis of previous experience in our laboratory. The experiments were performed and analyzed in a non-randomized and non-blinded fashion. No data were excluded from the analysis. Variance was similar between the groups that were statistically compared.

Additional information regarding human participants and animals used, real-time validation and availability, statistics, software for data analysis and reproducibility of results can be found in the **Life Sciences Reporting Summary** accompanying this manuscript.

**Additional Methods.** Methods for real-time PCR, PCR cloning, retrovirus-mediated gene expression and selection, electroporation-mediated gene silencing, western blot analysis, coimmunoprecipitation and the luciferase assay are available in the **Supplementary Note**

**Data availability.** The data supporting the findings of this study are available from the corresponding author upon reasonable request. The microarray data can be accessed at the Gene Expression Omnibus (GEO) repository ([GSE94527](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE94527)). Primer sets for real-time PCR, probes for qPCR by the TaqMan system and shRNA target sequences are available in **Supplementary Tables 3, 4 and 5**, respectively. Uncropped western blots, along with molecular weight standards, are included in **Supplementary Fig. 7**.

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## Life Sciences Reporting Summary

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For further information on the points included in this form, see [Reporting Life Sciences Research](#). For further information on Nature Research policies, including our [data availability policy](#), see [Authors & Referees](#) and the [Editorial Policy Checklist](#).

### ► Experimental design

#### 1. Sample size

Describe how sample size was determined.

Samples sizes were chosen based on previous experience in our laboratory.

#### 2. Data exclusions

Describe any data exclusions.

No data were excluded from the analysis.

#### 3. Replication

Describe whether the experimental findings were reliably reproduced.

All attempts at replication were successful.

#### 4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

The experiments were performed and analyzed in non-randomized fashions.

#### 5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

The experiments were performed and analyzed in non-blinded fashions.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

#### 6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

- |                          |                                                                                                                                                                                                                                          |
|--------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| n/a                      | Confirmed                                                                                                                                                                                                                                |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The <u>exact sample size</u> ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)                                    |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly                                         |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A statement indicating how many times each experiment was replicated                                                                                                                                 |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A description of any assumptions or corrections, such as an adjustment for multiple comparisons                                                                                                      |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The test results (e.g. $P$ values) given as exact values whenever possible and with confidence intervals noted                                                                                       |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A clear description of statistics including <u>central tendency</u> (e.g. median, mean) and <u>variation</u> (e.g. standard deviation, interquartile range)                                          |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Clearly defined error bars                                                                                                                                                                           |

See the web collection on [statistics for biologists](#) for further resources and guidance.

### ► Software

Policy information about [availability of computer code](#)

#### 7. Software

Describe the software used to analyze the data in this

Statistic analysis: GraphPad Prism 5

study.

Blotting Image Quantification: Image Quant TL (GE Healthcare Life Sciences)  
DNA microarray data: Analyzed with Feature Extraction software (Agilent Technologies) and normalized by 75 percentile shift using GeneSpring GX software (Agilent Technologies)

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* [guidance for providing algorithms and software for publication](#) provides further information on this topic.

## ► Materials and reagents

Policy information about [availability of materials](#)

### 8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

Monoclonal mouse anti-PAF-AH2 (T110) antibody was produced in our laboratory. Monoclonal mouse anti-FcεR1β (clone: JRK) antibody was kindly provided by J. Rivera (National Institutes of Health).

### 9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Antibodies to Syk (sc-1077), Lyn (sc-15), Fyn (sc-16) and Fgr (sc-74542) were from Santa Cruz Biotechnology. Antibodies to phosphorylated (p)-Syk Tyr525/526 (#2711), p-Gab2 Tyr452 (#3881), Gab2 (#3239), p-Lyn Tyr507 (#2731), Csk (#4980), p-Src Tyr416 (#2101) and Srcin1 (#3757) were purchased from Cell Signaling Technology. An antibody to Hck (ab75839) was from Abcam. An antibody to GAPDH (6C5) was from Calbiochem. An antibody to phosphotyrosine (4G10) was from Upstate Biotechnology. Antibodies to p-Fyn Tyr530 (SAB4503872) and PAF-AH2 (HPA018157) were from Sigma Aldrich.

### 10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

HEK293T cells were obtained from ATCC.

b. Describe the method of cell line authentication used.

HEK293T cells were not authenticated.

c. Report whether the cell lines were tested for mycoplasma contamination.

All cells are routinely tested for mycoplasma contamination.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by [ICLAC](#), provide a scientific rationale for their use.

Not applicable.

## ► Animals and human research participants

Policy information about [studies involving animals](#); when reporting animal research, follow the [ARRIVE guidelines](#)

### 11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Pafah2<sup>-/-</sup>, Pla2g4a<sup>-/-</sup> and Pnpla8<sup>-/-</sup> mice were backcrossed to C57BL/6 mice for 12 generations or to BALB/c background for 9 generations. All experiments using knockout mice (male, 12-14-week-old) were compared with their age-matched littermate control mice or wild-type mice purchased from CLEA Japan. MC-deficient Kit mutant mice (C57BL/6J-KitW<sup>-sh</sup>/W<sup>-sh</sup>) were purchased from the Jackson Laboratories. All mice were housed in climate-controlled (23 °C) specific pathogen-free facilities with a 12-h light-dark cycle, with free access to standard laboratory food (CE2; CLEA) and water. All animal experiments were performed in accordance with protocols approved by Animal Committees of the University of Tokyo and the Tokyo Metropolitan Institute of Medical Science in accordance with the Standards Relating to the Care and Management of Experimental Animals in Japan.

Policy information about [studies involving human research participants](#)

### 12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Human tissue was obtained during surgery (synovial tissues were obtained from patients with osteoarthritis who were undergoing joint replacement surgery; skin tissues, cord blood and peripheral blood were obtained from healthy donors; macroscopically normal lung tissues were obtained from patients with lung cancer) at the Nihon University Hospital under approval of the Ethics Committees of Nihon University School of Medicine, and all subjects provided written informed consent in accordance with the Helsinki Declaration of the World Medical Association.