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PII: S0008-8749(16)30035-1

DOI: <http://dx.doi.org/10.1016/j.cellimm.2016.05.005>

Reference: YCIMM 3542

To appear in: *Cellular Immunology*

Received Date: 13 June 2015

Revised Date: 16 May 2016

Accepted Date: 25 May 2016



Please cite this article as: T. Yang, Z. Xie, H. Li, L. Yue, Z. Pang, A.J. MacNeil, M.L. Tremblay, J-T. Tang, T-J. Lin, Protein tyrosine phosphatase 1B (PTP1B) is dispensable for IgE-mediated cutaneous reaction *in vivo*, *Cellular Immunology* (2016), doi: <http://dx.doi.org/10.1016/j.cellimm.2016.05.005>

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Protein tyrosine phosphatase 1B (PTP1B) is dispensable for IgE-mediated cutaneous reaction *in vivo*.

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Running title: PTP1B regulates IgE-mediated STAT5 in mast cells.

Abbreviations: BMMC, bone marrow-derived mast cell; BSA, bovine serum albumin; FBS, Fetal bovine serum; TNP, Trinitrophenyl; DNFB, dinitrofluorobenzene; DNP, dinitrophenyl; PTP1B, Protein tyrosine phosphatase 1B; STAT5, Signal Transducer and Activator of Transcription 5; OVA, ovalbumin; SCF, stem cell factor; HBSS, Hank's Balanced Salt Solution; PGE2, Prostaglandin E2; IκB, inhibitor of NF-κB; AKT, Protein kinase B; ELISA, enzyme-linked immunosorbent assay; ERK, extracellular signal-regulated kinase; IL, interleukin; JNK, c-Jun NH2-terminal kinase; MAPK, mitogen-activated protein kinase; NFAT, nuclear factor of activated T cells; TNP, trinitrophenyl; TNF, tumour necrosis factor; JAK, Janus Kinase; TYK, Tyrosine kinase; RTK, receptor tyrosine kinase.

Abstract

Mast cells play a critical role in allergic reactions. The cross-linking of FcεRI-bound IgE with multivalent antigen initiates a cascade of signaling events leading to mast cell activation. It has been well-recognized that cross linking of FcεRI mediates tyrosine phosphorylation. However, the mechanism involved in tyrosine dephosphorylation in mast cells is less clear. Here we demonstrated that protein tyrosine phosphatase 1B (PTP1B)-deficient mast cells showed increased IgE-mediated phosphorylation of the signal transducer and activator of transcription 5 (STAT5) and enhanced production of CCL9 (MIP-1γ) and IL-6 in IgE-mediated mast cells activation *in vitro*. However, IgE-mediated calcium mobilization, β-hexosaminidase release (degranulation), and phosphorylation of IκB and MAP kinases were not affected by PTP1B deficiency. Furthermore, PTP1B deficient mice showed normal IgE-dependent passive cutaneous anaphylaxis and late phase cutaneous reactions *in vivo*. Thus, PTP1B specifically regulates IgE-mediated STAT5 pathway, but is redundant in influencing mast cell function *in vivo*.

Introduction

Mast cells play a central role in allergic inflammation. Cross-linking of the FcεRI on mast cells activates multiple signaling pathways [1]. FcεRI is a tetrameric complex (αβγ₂) on mast cell surface [2]. Upon activation, the FcεRI β and γ subunits are phosphorylated at ITAMs by Src family protein tyrosine kinases [3, 4], leading to activation of a large number of downstream signaling molecules [3, 5, 6]. Protein tyrosine kinases (PTK) and protein Ser/Thr kinases play an essential role in FcεRI-mediated mast cell responses. The early molecular events immediately following FcεRI aggregation are mediated by PTK, such as Lyn, Fyn and Syk, and followed by activation of many Ser/Thr kinases such as PKC, Akt, and IκB or the Tyr/Thr kinase MAPK [3].

Cells utilize a coordinated balance between the protein kinases and phosphatases to transduce signals. Much of the research on FcεRI signaling has been focused on protein kinases. Equally important is the dephosphorylation of these proteins by protein phosphatases [7]. The coordinated actions of PTKs and protein tyrosine phosphatases (PTPs) determine the level of tyrosine phosphorylation. The important role of PTPs such as CD45 [8, 9], PTPα [10], Shp2 [11] or PTP-PEST [12] has been demonstrated in FcεRI-induced mast cell activation and allergic responses.

Protein tyrosine phosphatase 1B (PTP1B) is a 50 kDa non-transmembrane protein tyrosine phosphatase [13] and is localized predominantly to the endoplasmic reticulum [14]. A large number of PTP1B substrates have been identified. These substrates include cell surface receptors such as EGFR [15], insulin receptor (IR) [16] and a range of intracellular signaling molecules such as c-Src [17], STAT5 [18], JAK2 [19], p38 MAPK [20] or the linker for activation of T-cell (LAT) [21]. Interestingly, many of these potential PTP1B substrates such as LAT, STAT5, or p38 MAPK are also expressed in mast cells and have been recognized as contributors to IgE-mediated signaling in mast cells. However, a role of PTP1B in mast cells has not been studied previously. Given that PTP1B has been implicated in the pathogenesis of

allergic inflammation [22-24], we reason that PTP1B may regulate mast cell activation.

Here, we found that PTP1B deficient mast cells showed increased IgE-mediated phosphorylation of the STAT5 and enhanced production of CCL9 and IL-6. However, PTP1B deficient mast cells showed normal calcium mobilization, degranulation, and phosphorylation of Lyn, I κ B and MAPK, suggesting that IgE-mediated early molecular events are not affected by PTP1B. Interestingly, PTP1B deficient mice showed normal IgE-dependent passive cutaneous anaphylaxis and late phase cutaneous reactions *in vivo*. Thus, PTP1B specifically regulates IgE-mediated STAT5 pathway, but is redundant in influencing mast cell function *in vivo*.

Materials and Methods

Animals

PTP1B heterozygous (+/-) mice (BALB/c background) were provided by Michel Tremblay (McGill University, Montreal, QC, Canada). PTP1B heterozygous (+/-) mice were bred and genotyped by PCR. PTP1B deficient (PTP1B^{-/-}) and wild-type PTP1B^{+/+} mice from the same littermates were used. The protocols were approved by the University Committee on Laboratory Animals, Dalhousie University, in accordance with the guidelines of the Canadian Council on Animal Care.

Antibodies

Antibodies to PTP1B were purchased from Millipore (Bedford, MA). Antibodies to phospho-Akt (Ser473), AKT, phospho-ERK1/2, ERK, phospho-p38 (Thr180/Tyr182), phospho-JNK (Thr 183/Tyr 185), JNK, phospho-Lyn (Tyr507), Lyn, phospho-I κ B α (Ser32), I κ B α , phospho-STAT5 (Tyr694) and STAT5 were obtained from Cell Signaling Technology (Danvers, MA). Antibodies to p38 MAPK, actin, and HRP-linked secondary antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). FITC-conjugated rat anti-mouse c-kit mAb and FITC-rat IgG2a were purchased from Cedarlane Laboratories Ltd. (Hornby, ON).

FITC-conjugated rat anti-mouse IgE (IgG1) and FITC-rat IgG1 were purchased from BD Biosciences (San Jose, CA).

Mast cell culture, activation, and degranulation

Mouse bone marrow-derived mast cells (BMMCs) were cultured in a complete medium as previously described [25], supplemented with 35 ng/ml SCF (PeproTech, Rocky Hill, NJ). Mast cells were confirmed by toluidine blue staining and flow cytometry analysis for c-kit and IgE receptor expression. Following 5 - 6 weeks in culture mast cell purity was >98%. BMMCs were passively sensitized with IgE from TIB-141 cells (American Type Culture Collection). The following day, unbound IgE was removed by washing cells with RPMI 1640. Cells were then activated by stimulation with 10 ng/ml trinitrophenyl (TNP)-BSA (Biosearch Technologies, Novato, CA) for 20 min. Mast cell degranulation was determined by measuring β -hexosaminidase release.

Intracellular calcium

Sensitized BMMCs were loaded with 4 μ g/ml of Fura-2 AM (Invitrogen, Carlsbad, CA) for 30 minutes in the dark. After washing, cells were resuspended in HBSS buffer supplemented with 0.1% FBS at a concentration of 1×10^6 cells/ml. Fluorescence was measured by placing 2 ml of the cell suspension in a quartz cuvette with magnetic stirring in a RF-1501 spectrofluorophotometer (Shimadzu Co., Tokyo, Japan).

Western blotting

BMMCs were lysed in radioimmunoprecipitation assay buffer supplemented with a cocktail of protease and phosphatase inhibitors. Lysates (30 μ g) were subjected to electrophoresis in 10% SDS-polyacrylamide gels. Gels were transferred to polyvinylidene difluoride (PVDF) membrane, blocked with 5% non-fat milk powder, probed with primary and secondary antibodies, and detected by an enhanced

chemiluminescence detection system (Western Lightning Plus-ECL; PerkinElmer) on BioMax film (Kodak). Blots were scanned and quantified using ImageJ software.

Enzyme-linked Immunosorbent Assays (ELISA)

ELISA was used to measure cytokine and chemokine concentrations in cell-free supernatant according to the manufacturer's protocol (Invitrogen, Carlsbad, CA). Antibodies and standards (DuoSet) were from R&D Systems (Minneapolis, MN).

IgE-mediated passive cutaneous anaphylaxis

Mice were sensitized by intradermal injection of 20 ng anti-dinitrophenyl (DNP) IgE mAb (Sigma-Aldrich, St. Louis, MO) into left ear and foot. Saline was applied to right ear and foot as control. After 24 h, mice were challenged by i.v. injection of 100 µg DNP-BSA in 200 µl of Evan's blue dye (1% wt/vol; Sigma-Aldrich). Thirty minutes later, ear and foot was collected in 300 µl formamide (Sigma-Aldrich) and incubated at 80°C for 2 h to extract the Evan's blue dye. The absorbance was determined at 620 nm.

IgE-dependent late phase cutaneous reactions

Mice were passively sensitized by i.v. injection of 2 µg of anti-DNP IgE mAb (Sigma-Aldrich). After 24 h, a cutaneous reaction was elicited by the application of 20 µl dinitrofluorobenzene (DNFB, 1% w/v; Sigma-Aldrich) in acetone/olive oil (4:1) to both sides of the right hind paw or right ear, and 20 µl of acetone/olive oil to the left hind paw or left ear as a control. The thickness of the foot pad or ear was measured using a digital micrometer after 24 h. The weight of the hind paw or ear punch (8 mm) was also measured. The thickness and weight of right ear or right hind paw were used as baseline values. The DNFB-induced change in tissue thickness and weight was expressed as a percentage of the baseline values.

Statistical Analysis

Data are expressed as means $\pm$ SEM. Differences between different groups of animals or different mast cell populations (wild-type versus PTP1B deficient BMMC) were

evaluated using an unpaired Student t test. Two-way ANOVA followed with Tukey's tests were performed in experiments in which there were multiple groups (multiple time points) and there were two cell populations (wild-type and PTP1B deficient BMMCs). Differences were considered significant at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Statistical analysis was performed using IBM SPSS Statistics 22.0 (SPSS, Chicago, USA).

Result

PTP1B is not required for mast cell development

PTP1B deficient mast cells were obtained by culturing bone marrow cells from PTP1B deficient mice for 5 weeks. Toluidine blue staining showed that PTP1B deficient and wild-type mast cells have similar metachromatic staining and morphology (Fig. 1A). To confirm the absence of PTP1B in BMMCs, Western blotting was performed on total lysates from PTP1B-deficient and wild-type BMMCs. PTP1B was not detected in PTP1B-deficient BMMCs (Fig. 1B).

BMMCs were analyzed by flow cytometry for c-Kit and IgE receptor expression. No difference of c-Kit and IgE receptor expression was observed between PTP1B deficient and wild-type mast cells (Fig. 1C). These results indicate that mast cells develop normally *in vitro* in the absence of PTP1B.

To determine whether PTP1B is required for mast cell development *in vivo*, back skin, tongue, ear and intestine tissues from wild-type or PTP1B-deficient mice were fixed and stained by toluidine blue for mast cells. Consistent with *in vitro* studies, similar number of mast cells in the back skin, tongue, ear and intestine was observed in wild-type and PTP1B-deficient mice (Fig 1D, E).

PTP1B deficiency does not affect calcium mobilization or mast cell degranulation

IgE-mediated calcium mobilization is one of the key signaling event in mast cell activation [26]. To examine whether PTP1B modulates IgE-mediated calcium

mobilization, PTP1B-deficient BMMCs were sensitized with anti-TNP IgE, and loaded with Fura-2AM followed by stimulation with TNP-BSA. Calcium influx following TNP stimulation showed no difference between wild type and PTP1B-deficient BMMCs (Fig. 2A).

To investigate the role of PTP1B in FcεRI-mediated mast cell degranulation, wild-type and PTP1B-deficient BMMCs were sensitized with anti-TNP IgE and stimulated with TNP-BSA for 20 minutes. Mast cell degranulation was assessed by measuring the release of β-hexosaminidase. PTP1B deficiency showed no effect on mast cell degranulation *in vitro* (Fig. 2B). An *in vivo* model of mast cell degranulation in PTP1B-deficient and wild-type mice was conducted. Mice were passively sensitized with 200 ng anti-DNP IgE intradermally in ear and foot for 24 h followed by intravenous challenge with DNP-BSA in a 1% solution of Evans blue dye by tail vein injection. Thirty minutes later, ear and foot were collected for Evans blue dye extraction. The dye leakage was assessed by spectrophotometry. No significant difference in vascular permeability in ear tissue was observed between wild type and PTP1B-deficient mice (Fig. 2C). These results suggest that PTP1B does not affect FcεRI-mediated mast cell degranulation *in vitro* or *in vivo*.

PTP1B negatively regulated STAT5 phosphorylation following FcεRI-mediated BMMCs activation

To examine the effect of PTP1B deficiency on the IgE-mediated signalling pathways, wild-type and PTP1B-deficient BMMCs were sensitized with anti-TNP IgE, and stimulated with TNP-BSA. Cell lysates were analyzed by Western blotting for phosphorylation of lyn, AKT, IκB, MAPK, or STAT5. Phosphorylation of STAT5 was significantly enhanced after 3 h stimulation in PTP1B-deficient BMMCs, whereas phosphorylation of lyn, AKT, IκB, ERK, JNK, or p38 MAPK was not affected by PTP1B deficiency (Fig. 3).

PTP1B contributes to CCL9 and IL-6 production in response to IgE-mediated BMMCs activation.

IgE-mediated mast cell activation leads to production of various cytokines and chemokines. To determine whether PTP1B plays a role in IgE-mediated mast cell cytokine and chemokine production, PTP1B-deficient and wild-type BMMCs were sensitized with anti-TNP IgE and stimulated by TNP-BSA. Cell-free supernatants were collected and analyzed by ELISA for TNF, IL-6, CCL2, CCL3, CCL9, IL-4, IL-5 and IL-13 production. PTP1B deficient mast cells showed increased CCL9 levels at 3, 6, 12, and 24 h and increased IL-6 production at 3 h following TNP-BSA stimulation (Fig. 4). In contrast, IL-4 production was reduced in PTP1B deficient BMMCs, while no significant difference of TNF, CCL2, CCL3, IL-5 and IL-13 production was seen between PTP1B deficient and wild-type BMMCs (Fig 4).

To determine whether mast cells contain pre-formed cytokines, PTP1B deficient and wild-type BMMCs without TNP-BSA stimulation were lysed and used to determine IL-6, TNF, CCL9 and IL-4. None of these cytokines and chemokines was detectable in mast cell lysates [27].

To determine whether a different stimuli-mediated mast cell activation would be affected by PTP1B, PTP1B-deficient BMMC and wild-type BMMC were stimulated by phorbol 12-myristate 13-acetate (PMA, 100 nM) for 3 or 6 h, mast cell supernatants were used to determine IL-6, TNF and CCL9 production. PMA-mediated IL-6 and TNF production was similar between PTP1B-deficient BMMC and wild-type BMMC. PMA-mediated CCL-9 level was higher in PTP1B-deficient BMMC than that in wild-type BMMC [27].

PTP1B-deficiency does not impair IgE-mediated late phase cutaneous reaction in vivo

Mast cells contribute to the development of late phase allergic responses by production of cytokines and chemokines. To examine whether PTP1B is involved in IgE-mediated late-phase cutaneous reaction *in vivo*, PTP1B-deficient and wild-type mice were sensitized with anti-DNP IgE intravenously. After 24 h, a solution of 1% DNFB was applied epicutaneously to the ear and footpad. Antigen-specific edema was assessed by measuring of tissue weight and thickness. Interestingly,

PTP1B-deficient and wild-type mice displayed similar levels of tissue edema in the ear and footpad at 24 h after DNFB challenge (Fig. 5). Similarly, no significant differences of antigen-specific edema after 16 h and 32 h of DNFB challenge were observed between PTP1B-deficient and wild-type mice [27].

Discussion

Tyrosine phosphorylation has been recognized to be critical in many biological events including IgE-mediated mast cell activation. The cellular equilibrium of protein tyrosine phosphorylation is achieved through the actions of PTKs and PTPs. In the human genome, there are 107 genes that encode PTPs [28] and 90 genes that encode PTKs [29]. It is generally considered that PTPs and PTKs have specific and non-redundant functions [30]. The effects of PTKs in IgE-mediated immune response suggest that PTPs are involved in the IgE-mediated signaling events. Indeed, several PTPs including CD45 [8, 9], PTP α [10], Shp2 [11] and PTP-PEST [12] has been recognized in IgE-mediated mast cell activation. PTP1B is ubiquitously expressed and have been recognized in a number of signaling pathways. However, a role of PTP1B in mast cell biology has not been reported previously.

A number of early molecular events following IgE-induced Fc ϵ RI aggregation involve tyrosine phosphorylation. Tyrosine residues in the immunoreceptor tyrosine-based activation motifs (ITAMs) of the Fc ϵ RI β - and γ -chains are phosphorylated by Lyn [31], leading to tyrosine phosphorylation of Syk. Subsequently, Syk phosphorylates 4 tyrosine residues on the membrane-localized adaptor protein linker for activation of T cells (LAT) [32]. Once LAT is activated as a scaffold platform, signals begin to branch out into several pathways including calcium mobilization, MAP pathway activation and I κ B-NF κ B activation [33]. LAT is essential for IgE-mediated calcium mobilization and degranulation in mast cells [34]. To our surprise, PTP1B deficient mast cells showed normal IgE-mediated Lyn phosphorylation, calcium mobilization, and mast cell degranulation. These results

suggest that none of tyrosine phosphorylation events before calcium mobilization occurred in the early signaling stage following FcεRI aggregation are modulated by PTP1B. In neuron PTP1B has been shown to negatively regulate calcium mobilization [35]. In platelet PTP1B directly interacts with LAT upon activation by FcγRIIa cross-linking [21]. The lack of effect of PTP1B in IgE-mediated calcium mobilization and degranulation highlights the cell type specific role of PTP1B and the uniqueness of IgE-mediated signaling mechanism in mast cells. These results suggest that a role of PTP1B in IgE-mediated mast cell activation could not readily be extrapolated from its effects found in other cell types.

IgE-mediated STAT5 phosphorylation was enhanced in PTP1B deficient mast cells suggesting that PTP1B is involved in STAT5 dephosphorylation during IgE-mediated mast cell activation. This is consistent with the finding that STAT5 is a PTP1B substrate in breast cancer [36] and in epithelial cells [18]. Increased STAT5 phosphorylation may explain the enhanced CCL9 and IL-6 production in PTP1B deficient mast cells. However, we noted that STAT5 deficient mast cells show profound defects of mast cell function including degranulation, TNF production and survival [37]. In contrast, we found that PTP1B deficiency does not modulate mast cell development, TNF production or degranulation. Thus, although PTP1B may be involved in the dephosphorylation of STAT5 during IgE-mediated mast cell activation, it does not appear to be a major functional regulator for STAT5 in mast cells.

PTP1B has been shown as a regulator of MAPK activation in B cells [20], and PI3K/AKT activation in adipocytes [38]. In contrast, our western blot analysis revealed that these signaling pathways in FcεRI-dependent mast cells are not affected by PTP1B deficiency. These findings further support the notion that a role of PTP1B in mast cells could not be predicted based on its functions found in other cell types.

In an ovalbumin-mediated allergic asthma model in mice, PTP1B deficiency exacerbates cytokine production and accelerates leukocyte trafficking *in vivo* [24]. Given that mast cells are a central player in allergic inflammation, we examined whether PTP1B regulates mast cell function. We found that PTP1B deficiency has limited effects on selected chemokine and cytokine production (CCL9 and IL-6) and

has no effect on IgE-mediated cutaneous allergic inflammation *in vivo*. These results suggest that mast cells may not be a major contributor in the exacerbated inflammation seen in ovalbumin-induced allergic asthma of PTP1B deficient mice. Indeed, endothelial cell PTP1B was found to be a regulator for leukocyte recruitment during allergic inflammation [23].

In summary, PTP1B deficiency led to enhanced IgE-mediated STAT5 phosphorylation and increased CCL9/MIP-1 γ and IL-6 production by mast cells. However, mast cell degranulation, IgE-mediated passive cutaneous anaphylaxis and late phase cutaneous reactions in mice were not affected by PTP1B deficiency. These results suggest that PTP1B is not a major regulator in IgE-mediated mast cell activation despite the overwhelming evidence of PTP1B in a wide range of biological events such as cancer development and diabetes. Thus, mast cells may have redundant and overlapping PTPs in the regulation of IgE-mediated tyrosine phosphorylation.

Acknowledgement

This work is supported by a grant from Natural Science Foundation of China

(81471564) to TJL and a grant from Canadian Institutes of Health Research to TJL.

MLT is the holder of the Jeanne and Jean-Louis Levesque Chair in Cancer Research.

We would like to thank Fang Liu for her technical assistance with isolation of bone-marrow derived mast cells and IgE-dependent cutaneous reaction studies *in vivo*.

Authorship

Contributions: TY and TJL conceived and designed the experiments. TY and LY performed the experiments. TY, ZX, HL, AJM, JTT and TJL analyzed the data. TY and TJL wrote the paper. MLT contributed vital materials (PTP1B deficient mice).

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Figure Legends

Fig 1. PTP1B deficiency does not affect mast cell maturation *in vitro*. A. Bone marrow cells from PTP1B deficient and wild-type mice were cultured for 5 weeks in conditioned medium and stained with toluidine blue. A representative photomicrograph is shown (original magnification $\times 100$). B. Western blot analysis of total protein lysates of mouse bone marrow-derived mast cells (BMMC) for PTP1B. No PTP1B proteins were detectable in PTP1B deficient mast cells. C. BMMCs from PTP1B deficient and wild-type mice were stained with FITC-conjugated anti-c-Kit Ab or anti-IgE Ab, and examined by flow cytometry. D and E. Back skin, tongue, ear and intestine tissues from wild-type or *PTP1B*-deficient mice were fixed and stained by toluidine blue for mast cells. Specimens were viewed on a microscope (Eclipse E600; Nikon) equipped with a camera (DMX1200; Nikon) and a DDL 40 $\times/0.75$ objective lens. Acquisition was performed with Nikon ACT-1 software (version 2.20). The number of mast cells was enumerated by counting 5 randomly selected fields with a 40 $\times/0.75$ objective lens. Data are means $\pm$ SEM ($n = 5$ fields). Bars, 10 μm .

Fig 2. PTP1B deficiency does not affect IgE-mediated calcium mobilization and mast cell degranulation. A. Intracellular calcium ion flux analysis of PTP1B deficient and wild-type BMMCs following IgE-mediated mast cell activation. B. β -Hexosaminidase release by stimulated PTP1B deficient and wild-type mast cells shows no difference in mast cell degranulation *in vitro*. C. Passive cutaneous anaphylaxis in wild-type and PTP1B deficient mice by Evan's blue dye leakage in ear and foot tissue. Mice were intradermally injected with 20 ng of anti-DNP IgE in the ear tissue. After 24 h, mice received i.v. injection of 100 mg DNP-BSA in 1% w/v Evan's blue dye. Ear punches (8 mm) and foot pads were collected 30 min later, and Evan's blue dye was extracted from ear and foot tissue. Samples were measured for dye leakage into tissue at 620 nm ($n = 8$ mice).

Fig 3. PTP1B deficiency induces increased STAT5 activation but not MAPKs, AKT, and I κ B α . PTP1B deficient and wild-type BMMCs were sensitized with anti-TNP IgE and stimulated for various lengths of time with TNP-BSA *in vitro*. Total lysates were analyzed by Western blot for phosphorylated (p-) and total STAT5, AKT, ERK, p38, JNK, I κ B α and lyn. An increase in STAT5 phosphorylation was observed in PTP1B deficient mast cells. Actin served as a loading control. A, Representative blot of STAT5. B, Relative STAT5 phosphorylation ($n = 4$ separate experiments). C, Representative blots of ERK, JNK, p38, AKT, I κ B α and lyn. D, Relative ERK phosphorylation ($n = 3$ separate experiments). E, Relative JNK phosphorylation ($n = 3$ separate experiments).

Fig 4. PTP1B differentially regulates IgE-dependent IL-4, CCL9, and IL-6 expression. PTP1B deficient and wild-type BMMCs were sensitized with anti-TNP IgE and stimulated for various lengths of time with TNP-BSA *in vitro*. TNF (A), IL-6 (B), CCL2 (C), CCL3 (D), CCL9 (E), IL-4 (F), IL-5 (G) and IL-13 (H) production was assessed by ELISA in cell-free culture media following TNP-BSA stimulation. Data are expressed as mean $\pm$ SEM (n = 4 separate experiments). *p < 0.05, PTP1B +/+ versus PTP1B -/-.

Fig 5. PTP1B deficiency shows no deficit in late-phase cutaneous anaphylaxis. PTP1B deficient and wild-type mice were sensitized by i.v. injection of 2 μ g of anti-DNP IgE mAb. Twenty-four hours later, 1% DNFB was applied to both the left and right ears and hind feet. The following day, ear weight (A) and thickness (B) as well as the foot weight (C) and thickness (D) were measured. Data are expressed as mean $\pm$ SEM (n = 12 mice in each group).

Fig 1

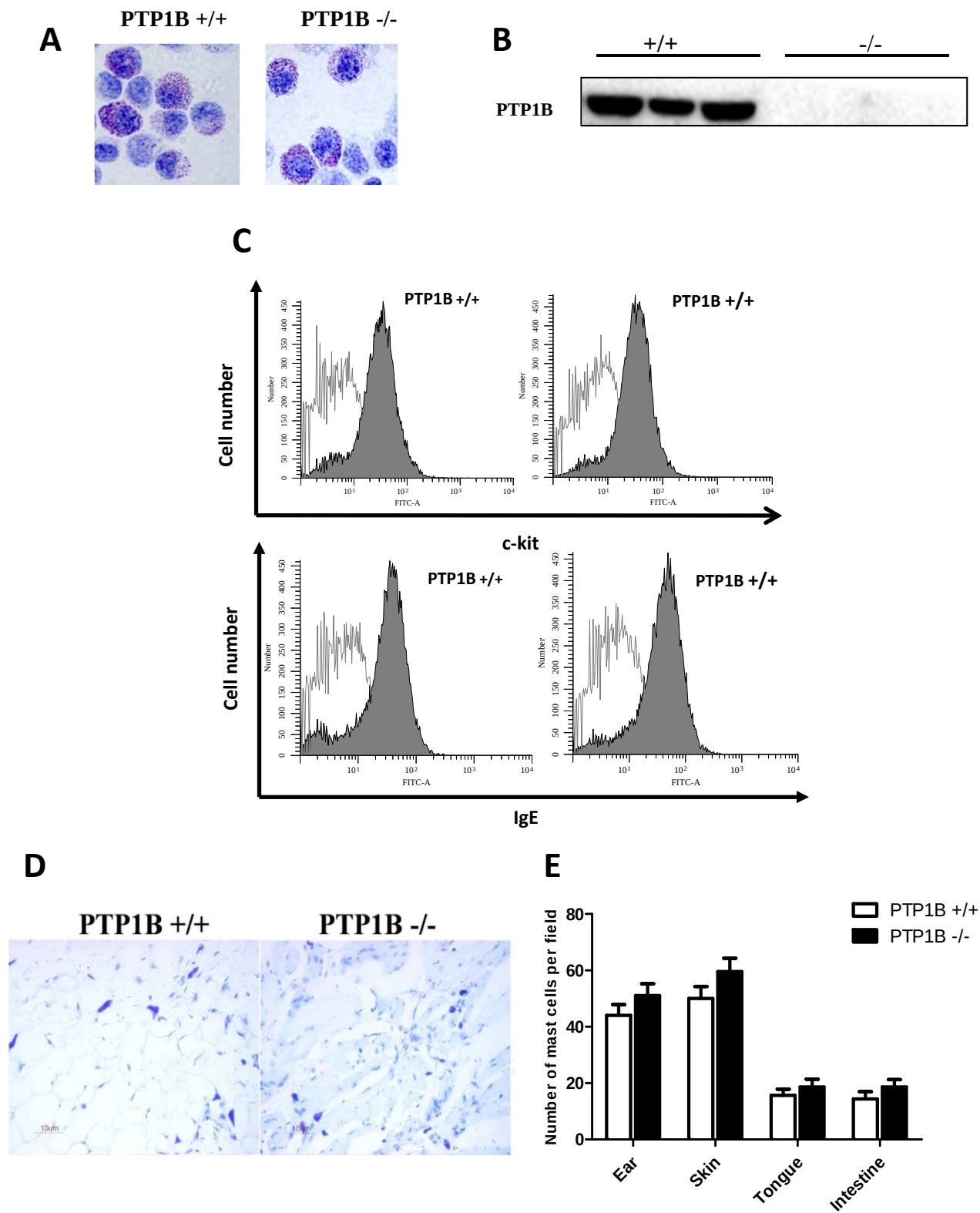


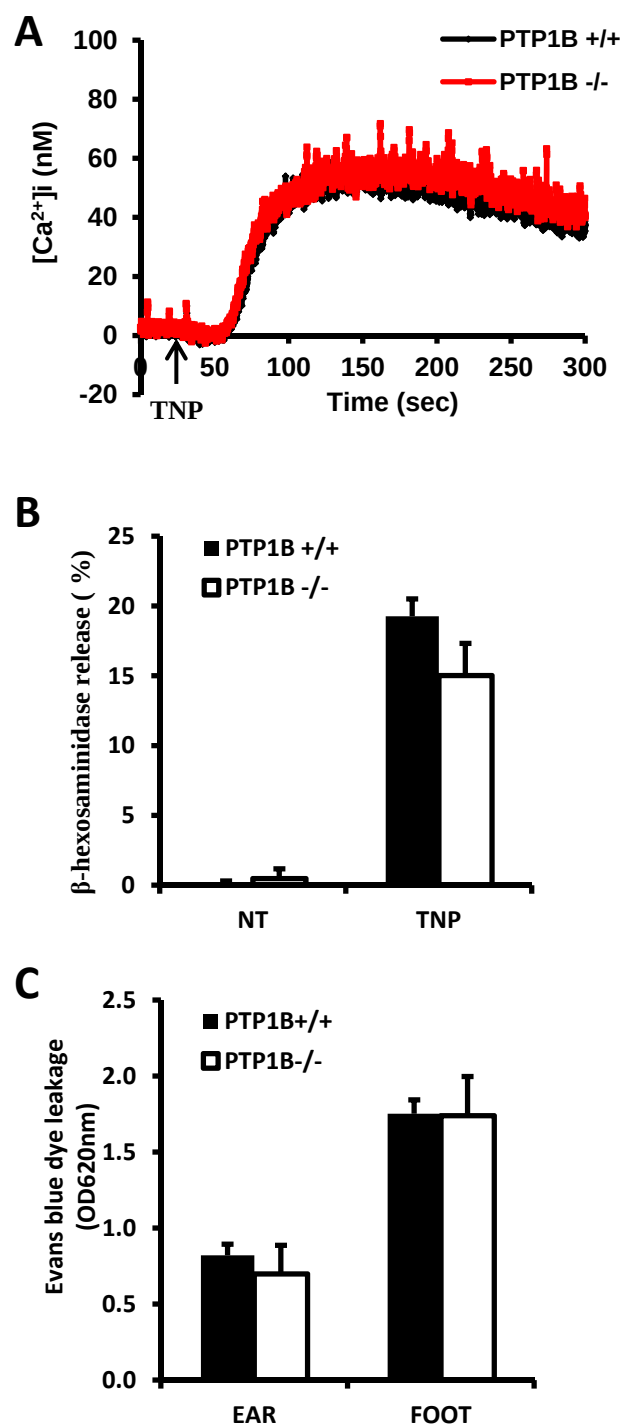
Fig 2

Fig 3

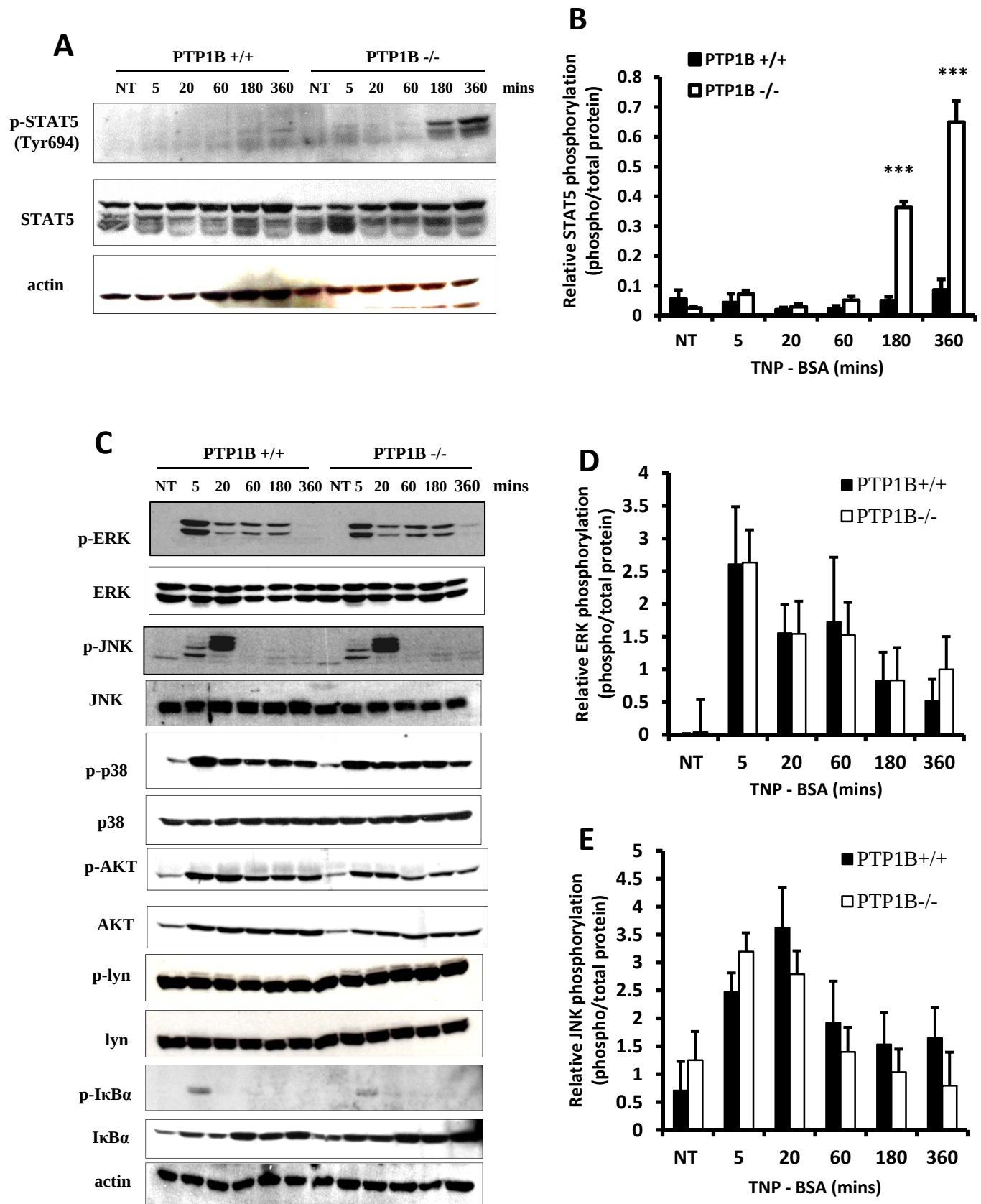


Fig 4

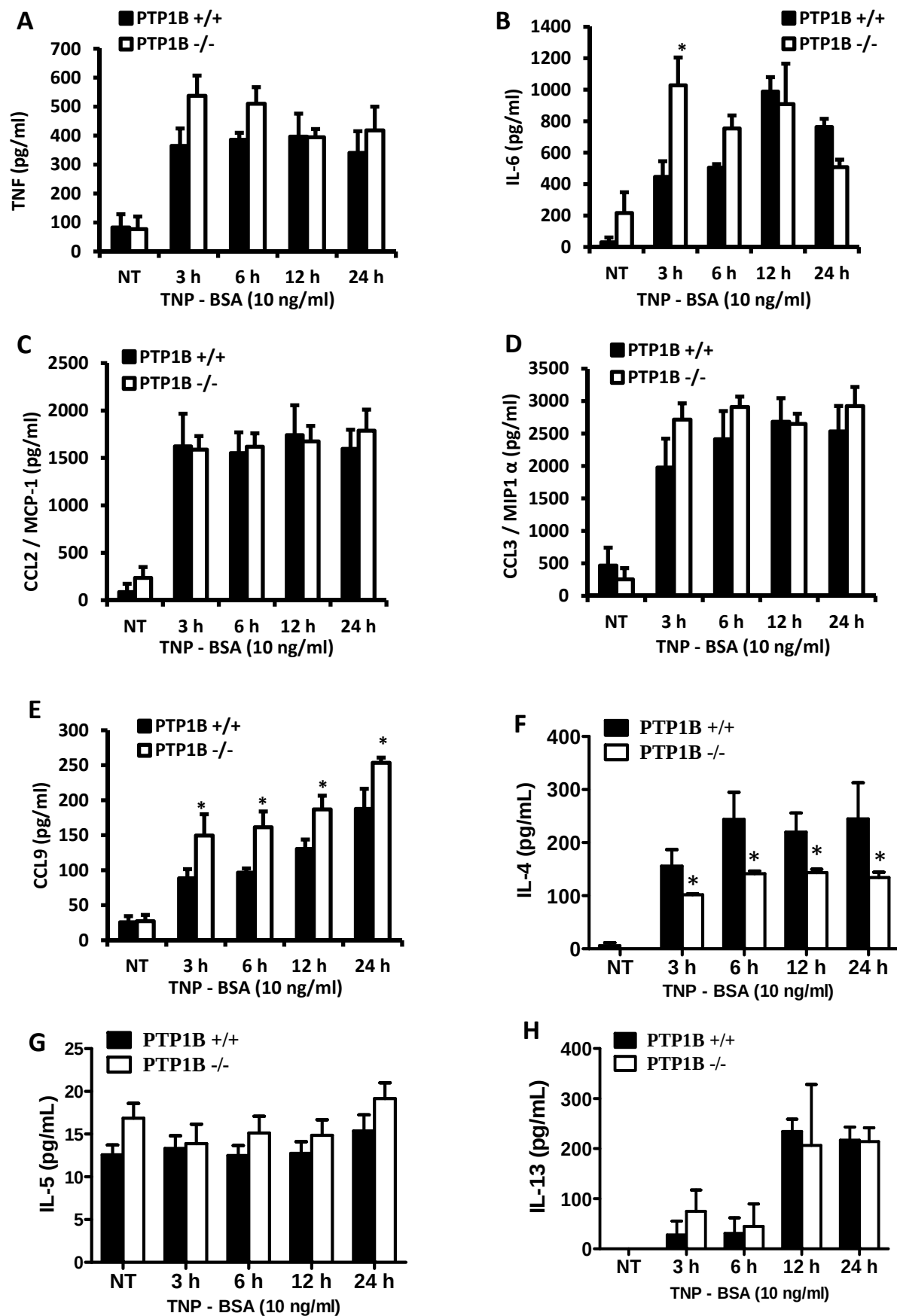
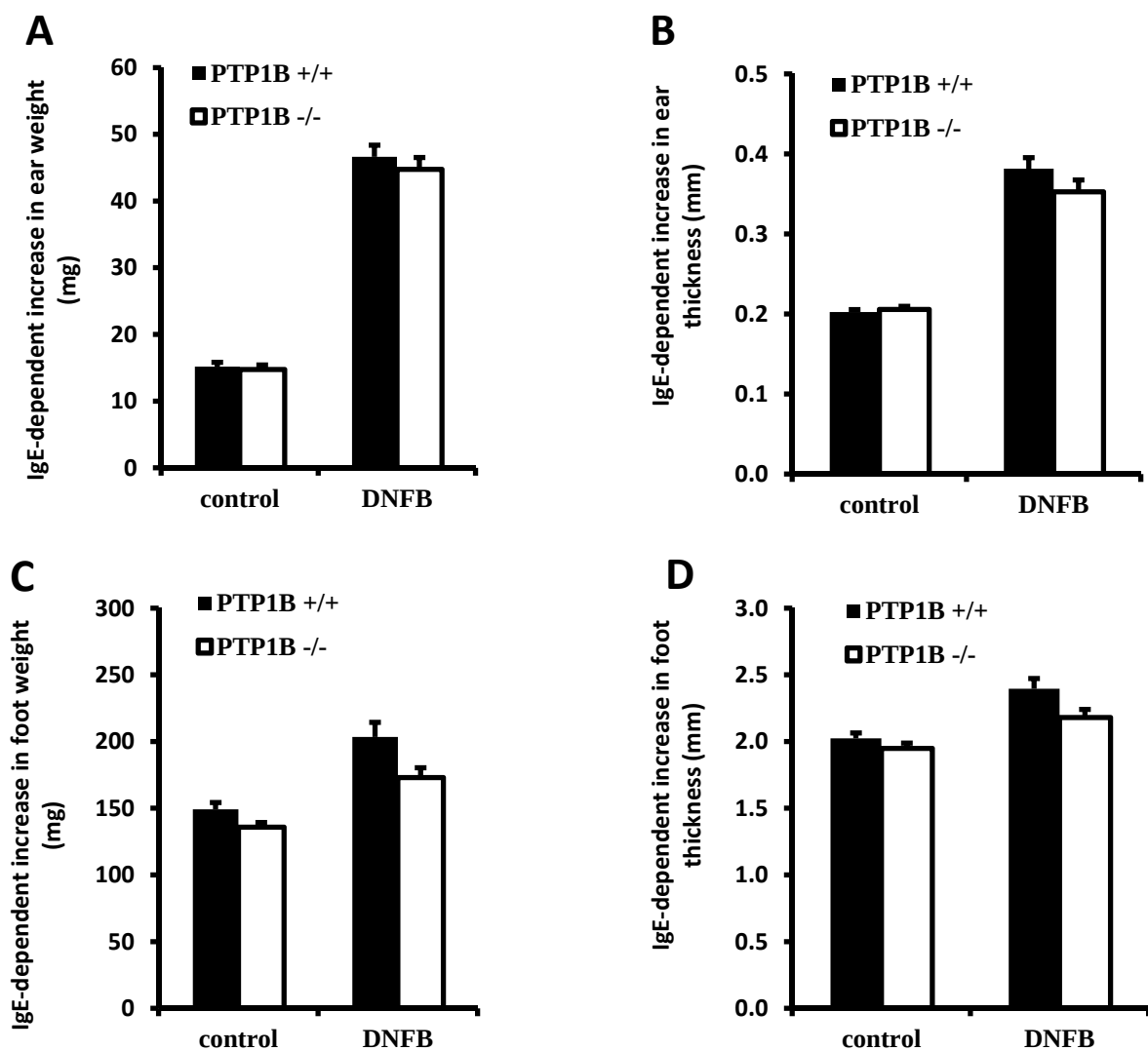


Fig 5

Highlights

- PTP1B differentially regulates IgE-mediated CCL9 and IL-4 production by mast cells *in vitro*.
- PTP1B is redundant for IgE-mediated calcium flux and degranulation *in vitro*.
- PTP1B is redundant for IgE-mediated cutaneous reaction *in vivo*.