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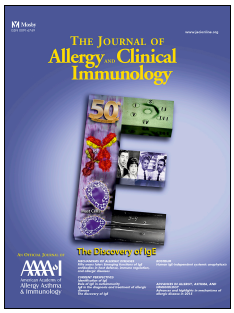

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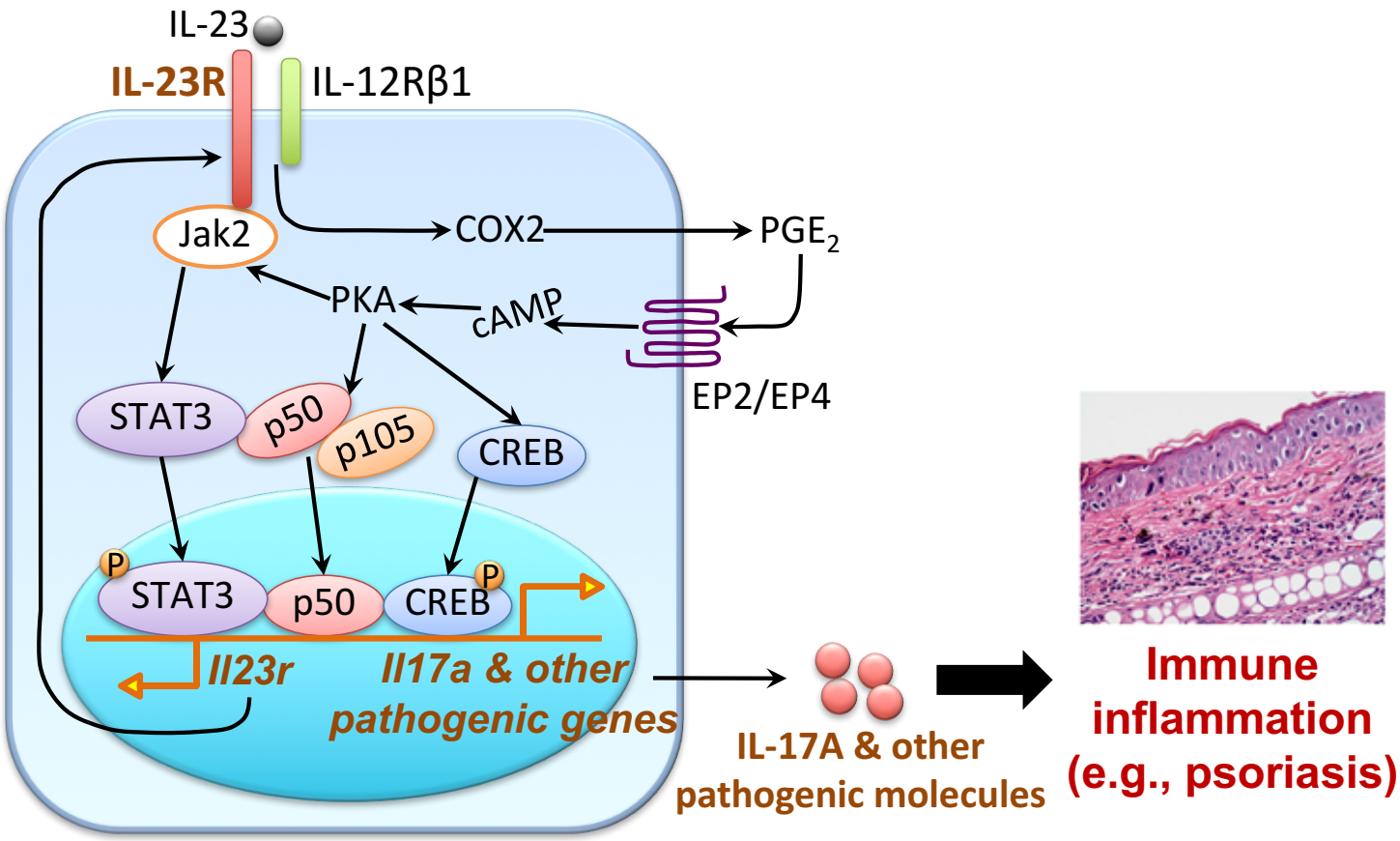
Intrinsic PGE_2 drives Th17 pathogenicity.



PGE_2, Prostaglandin E_2; **COX2,** cyclooxygenase 2; **cAMP,** cyclic adenosine monophosphate; **PKA,** Protein kinase A; **CREB,** cAMP response element binding protein; **Jak2,** Janus kinase 2; **STAT3,** Signal transducer and activator of transcription 3.

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ABSTRACT

Background: Interleukin-23 (IL-23) is the key cytokine for generation of pathogenic IL-17-producing helper T (Th17) cells that critically contribute to autoimmune diseases. However, how IL-23 generates pathogenic Th17 cells remains to be elucidated.

Objectives: To examine the involvement, molecular mechanisms and clinical implications of prostaglandin (PG) E_2-EP2/EP4 signaling in induction of IL-23-driven pathogenic Th17 cells.

Methods: The role of PGE_2 in induction of pathogenic Th17 cells was investigated in mouse Th17 cells in culture *in vitro* and in IL-23-induced psoriasis mouse model *in vivo*. Clinical relevance of findings in mice was examined by gene expression profiling of IL-23 and PGE_2-EP2/EP4 signaling in psoriatic skin from patients.

Results: IL-23 induces *ptgs2* encoding cyclooxygenase 2 in Th17 cells and produces PGE_2, which acts back on PGE receptors EP2 and EP4 in these cells and enhances IL-23-induced expression of an IL-23 receptor subunit gene, *Il23r*, by activating STAT3, CREB1 and NF-κB through cAMP-protein kinase A signaling. This PGE_2 signaling also induces expression of various inflammation-related genes, which possibly function in Th17 cell-mediated pathology. Combined deletion of EP2 and EP4 selectively in T cells suppressed accumulation of IL-17A$^+$ and IL-17A$^+$IFN-γ$^+$ pathogenic Th17 cells and abolished skin inflammation in IL-23-induced psoriasis mouse model. Analysis of human psoriatic skin biopsies shows positive correlation between PGE_2 signaling and the IL-23/Th17 pathway.

Conclusions: The T cell-intrinsic EP2/EP4 signaling is critical in IL-23-driven generation of pathogenic Th17 cells and consequent pathogenesis in the skin.

Key Messages

IL-23 triggers T cell-intrinsic PGE_2-EP2/EP4 signaling that is critical in Th17 cell-mediated immune pathogenesis.

The PGE_2-EP2/EP4 signaling functions synergistically with IL-23 and not only amplifies *Il23r* expression but also induces a unique pathogenic gene expression signature by activating STAT3, CREB1 and NF-κB.

This PGE_2 signaling can be a therapeutic target of Th17 cell-mediated diseases, because combined blockade of EP2 and EP4 suppresses IL-23-induced pathogenic Th17 cell generation and consequent psoriatic skin inflammation.

Capsule Summary

IL-23 mobilizes T cell-intrinsic PGE_2-EP2/EP4 signaling, which is critical in IL-23-induced pathogenic Th17 cell generation. Combined blockade of EP2 and EP4 suppressed IL-23-induced skin inflammation, suggesting this pathway as potential therapeutic target of Th17-mediated diseases.

Key words*: psoriasis, pathogenic Th17 cells, IL-23R, prostaglandin* E_2*, prostaglandin E receptor EP2, prostaglandin E receptor EP4, STAT3, CREB1, NF-κB*

Abbreviations used:

Th1, helper T cell type 1

Th2, helper T cell type 2

Th17, helper T cell type 17

Treg, regulatory T cell

PG, prostaglandin

COX, cyclooxygenase

cAMP, cyclic adenosine monophosphate

PKA, protein kinase A

CREB, cAMP Responsive Element Binding Protein

Epac, exchange factor directly activated by cAMP

db-cAMP, dibutyryl cAMP

IMQ, imiquimod

INTRODUCTION

$CD4^+$ T cells differentiate into Th1, Th2 and Th17 cells in response to specific cytokine milieu present in microenvironment of inflammation and mediate immune inflammatory responses in respective settings.[1-4] Among these Th subsets, Th17 cells mediate inflammatory responses in many autoimmune diseases including multiple sclerosis, inflammatory bowel diseases such as Crohn's disease, psoriasis and rheumatoid arthritis. The importance of Th17 cells in these processes was suggested first in animal models of these diseases including experimental autoimmune encephalomyelitis (EAE) and IL-23- or imiquimod-induced psoriasis model,[5-9] and validated recently by clinical effectiveness of antibodies targeting to IL-23 in patients with psoriasis.[10-14]

Differentiation of Th17 cells from naïve $CD4^+$ T cells is driven by the combined actions of interleukin-6 (IL-6) and transforming growth factor-β1 (TGF-β1).[15-19] However, differentiated Th17 cells have little capacity to induce autoimmune and inflammatory pathology.[20] It should be noted that these Th17 cells exhibit plasticity and could transdifferentiate into other effector T cell types or even regulatory T cells under certain context such as inflammation or autoimmune disease.[21-23] Accumulating evidences suggest that T cell intrinsic IL-23 signaling not only increases IL-17 production of Th17 cells but also plays a crucial role in inducing and stabilizing their pathogenicity.[20,24-27] It is known that IL-23 acts on IL-23 receptor composed of IL-23R and IL-12Rβ1, activates signal transducer and activator of transcription 3 (STAT3) and induces expression of *Il23r,* thus forming the self-amplification loop. The pathophysiological importance of this IL-23-IL-23 receptor signaling has been indicated by several genomic studies that showed positive correlation between single nucleotide

polymorphisms (SNPs) of genes involved in this pathway, e.g., *IL23R*, *IL12B (p40)*, *JAK2* and *STAT3*, and a wide range of IL-17-dependent autoimmune diseases.[28-30]

While it was shown that IL-23 signaling induces expression of Th17 pathogenic signature genes through activation of STAT3,[31,32] other transcription factor(s) besides STAT3 are also implicated for induction of pathogenic Th17 cells, because IL-6 that activates STAT3 similarly to IL-23 cannot induce IL-23R gene expression.[32] The identity of additional transcriptional factor(s) and regulatory mechanisms are therefore important issues to be defined. Moreover, how IL-23 cooperates with other inflammatory factors formed in disease microenvironment and how critical is such cooperation for pathogenic conversion of Th17 cells and overall pathology remain largely obscure. Clarification of these points could provide a new opportunity to develop small molecule drugs as therapeutic alternatives to anti-IL-23 antibodies without systemic immune suppression. Biological agents may additionally cause unpredictable adverse events[33] and can be costly on long-term use.[34] It should also be mentioned that JAK inhibitors that are now being evaluated in their efficacy in autoimmune diseases are presumably not free from adverse effects, either, because of their effects of general immune suppression.[35]

Prostanoids including prostaglandin (PG) D_2, PGE_2, $PGF_{2\alpha}$, PGI_2 and thromboxane A_2 (TXA_2) are oxygenated metabolites of arachidonic acid produced by sequential actions of cyclooxygenase (COX) and respective synthases, and act on their cognate receptors, DP for PGD_2, EP1 to 4 for PGE_2, IP for PGI_2, FP for $PGF_{2\alpha}$, and TP for TXA_2, to exert their actions.[36] While prostanoids were previously regarded as immunosuppressants,[37,38] recent studies have revealed their immunostimulatory actions in processes such as cytokine production, dendritic cell maturation, macrophage

activation, and differentiation and expansion of Th subsets.[39-41] Indeed, the PGE_2-EP2 and EP4 (EP2/EP4) signaling enhances Th1 differentiation by inducing the expression of an IL-12 receptor subunit, *Il12rb2*, and interferon-γ receptor, *Ifngr1*, thus facilitating IL-12 signaling and Th1 differentiation.[42,43] Notably, this PGE_2-EP2/EP4 signaling was also reported to synergize with IL-23 to facilitate Th17 cell expansion both in murine and human T cells.[43-45] However, whether the PGE_2-EP2/EP4 signaling is involved in induction of pathogenic Th17 cells, and, if so, how remains unknown.

In this study, we have examined how the PGE_2-EP2/EP4 signaling and IL-23 stimulation together regulate the generation of pathogenic Th17 cells. Through this analysis, we have identified the transcription mechanisms in addition to STAT3 that regulate *Il23r* expression and Th17 pathogenicity. We have further clarified the importance of the PGE_2 signaling in the Th17-mediated immune inflammation *in vivo*, and found the correlation between PGE_2-EP2/EP4 signaling and IL-23-IL-23 receptor signaling in biopsy samples from psoriasis patients.

METHODS

Mice

All animal experiments were approved by the Institutional Animal Care and Use Committee of Kyoto University Graduate School of Medicine, and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. C57BL/6NCrSlc mice were purchased from Shimizu laboratory, and Lck-Cre mice and B6.Cg-*Nfkb1*^{tm1Bal}/J mice were purchased from Jackson Laboratory. Mice deficient in *Ptger2*[46] and mice with floxed *Ptger2*[47] were established in our laboratory. Mice with floxed *Ptger4* was a kind gift of Richard Breyer.[48]

Psoriasis models

To induce psoriasis-like lesion in the ear in IL-23-induced psoriasis mouse model, mice were subcutaneously injected with IL-23 (500 ng; #130-096-677, Miltenyi, Bergisch Gladbach, Germany) once a day in one ear and with PBS in the contralateral ear as a control. In imiquimod (IMQ)-induced psoriasis mouse model, baselna cream containing 10% IMQ was applied onto the ear of mice once a day. Ear thickness was then measured by a digital micrometer (#KM-BMB1-25, Mitutoyo, Kawasaki, Japan) every other day. In some experiments, an antagonist for EP4, AS1954813,[49] suspended in 0.5 % methylcellulose was orally administered twice a day or indomethacin and SC-236 were administered by drinking water during the experimental period.

Other methods

See the Supplementary Methods section in this article's Online Repository at www.jacionline.org.

RESULTS

IL-23 mobilizes the endogenous COX-2-PGE_2-EP2/EP4 signaling that enhances induction of *Il23r* expression in Th17 cells

Given the previous findings[43-45] that the PGE_2-EP2/EP4 signaling enhances IL-23-induced Th17 cell expansion, we questioned here whether and how this signaling contributes to pathogenic Th17 cell generation by IL-23. To investigate this issue, we first cultured $CD4^+$ T cells from mouse spleen under the Th17-skewing condition (IL-6 plus TGF-β1) for 4 days, and then incubated with IL-23 for additional 3 days. Consistent with our previous findings,[43] the addition of PGE_2 to the latter culture significantly enhanced IL-23-induced expansion and *Il17a* expression of Th17 cells (Fig. 1, A and B). Interestingly, we also noted that PGE_2 markedly up-regulated IL-23-induced expression of *Il23r*, which was mimicked by both EP2- and EP4-selective agonists (Fig. 1, C). Since both EP2 and EP4 activates PKA and Epac by increasing intracellular cAMP,[36] we examined effects of compounds acting on these signaling, and found that a cAMP analogue, dibutyryl cAMP (db-cAMP), forskolin (FSK) and a phosphodiesterase inhibitor, IBMX, all synergized with IL-23 and significantly amplified IL-23-induced *Il23r* expression and IL-17A production in these cells (Fig. 1, D and E). Furthermore, the enhancement of *Il23r* expression was reproduced by a PKA agonist (N6-Bnz-cAMP, 300 μM) but not an Epac activator (8-pCTP-2'-O-Me-cAMP, 300 μM) (Fig. 1, F), and, consistently, was ameliorated by treatment with a PKA inhibitor (H-89, 10 μM) (Fig. 1, G).

Notably, IL-23 stimulation significantly increased *Ptgs2* (COX-2) gene expression in Th17 cells (Fig. 2, A) and produced subnanomolar concentrations of PGE_2

in the culture medium (Fig. 2, B). Moreover, incubation with non-selective COX inhibitor (indomethacin, 100 μM) or a selective COX-2 inhibitor (SC-236, 100 μM) but not a selective COX-1 inhibitor (SC-560, 100 μM) significantly blocked the induction of *Il23r* expression in response to both IL-23 alone and IL-23 and PGE_2 in combination (Fig. 2, C and Fig E1, A). In addition, antagonists selective to EP2 (PF-04418948) or EP4 (ONO-AE3-208) also suppressed *Il23r* expression (Fig. 2, D). Intriguingly indomethacin and SC-236 suppressed the expression of *Il23r* induced by IL-23 and PGE_2 to the level that these inhibitors achieved in the presence of IL-23 alone, suggesting that they cancelled the effect of exogenously added PGE_2 (Fig. 2, D and Fig E1, A). Given that PGE_2 added to the culture medium time-dependently degrades,[50] these results suggest that exogenously added PGE_2 induces COX-2 and produces PGE_2 endogenously and continuously as we reported previously,[51] which makes more contribution to *Il23r* induction, and that indomethacin and COX-2 inhibitor block this process. Indeed, the addition of stable EP2 and EP4 agonists overcame the *Il23r* suppression by indomethacin (Fig E1, B). Therefore, these data together suggest that IL-23 stimulates Th17 cells to produce PGE_2, which acts back to EP2 and EP4 on these cells to augment *Il23r* expression in a positive feedback manner.

Induction of *Il23r* expression by IL-23 and PGE_2-cAMP signaling is mediated through not only STAT3 but also CREB1 and NF-κB

We then investigated transcription factors responsible for induction of *Il23r* expression in Th17 cells by IL-23 and PGE_2-EP2/EP4 signaling. Because IL-23 activates STAT3 to induce *Il23r* expression,[52] we first examined the effect of a STAT3 inhibitor. The

addition of STAT3 inhibitor VII suppressed *Il23r* expression not only by IL-23, but also by db-cAMP, and both (Fig. 3, A), indicating that the db-cAMP action was also mediated by STAT3. Consistently, Y705 phosphorylation of STAT3 was increased by db-cAMP at 5 and 30 min (Fig E2, A), which were ameliorated not only by the addition of STAT3 inhibitor VII but also by H-89 (Fig. 3, B), indicating the involvement of PKA in db-cAMP-mediated Y705 phosphorylation of STAT3. Intriguingly, the Y1007/1008 phosphorylation of JAK2, a kinase responsible for STAT3 Y705 phosphorylation in Th17 cells, was enhanced by db-cAMP, and this enhancement was suppressed by Src Kinase Inhibitor I (Fig E2, B), indicating cAMP-PKA activates STAT3 through c-Src-JAK2 pathway.

Although the above findings demonstrated that IL-23 and PGE_2-cAMP signaling converge at STAT3 activation, it is well known that other STAT3 activators, such as IL-6 and IL-21, cannot substitute for IL-23 in the expansion of Th17 population,[32] indicating that STAT3 is not the sole transcription factor regulating expression of *Il23r*. Since PKA activates CREB1,[36] we investigated the involvement of CREB1 in *Il23r* expression. Both KG-501, a CREB1 inhibitor,[53] and RNAi for CREB1 suppressed *Il23r* induction in response to db-cAMP, IL-23 or both, suggesting the involvement of CREB1 in *Il23r* expression in Th17 cells **(**Fig. 3, C and D**)**. As IL-23 signaling enhances endogenous PGE_2 production via induction of COX-2 expression in Th17 cells (Fig. 2, A and B), the suppression of *Il23r* expression by inhibition or depletion of CREB1 could be due to inhibition of this endogenous PGE_2 signaling for *Il23r* induction.

Furthermore, we detected an increase in S536 phosphorylation of NF-κB p65 (pp65) in response to db-cAMP, IL-23 or both at 24 h (Fig. 3, E) and an increase in

S933 phosphorylation of NF-κB p105 subunit, a precursor of p50, in response to db-cAMP alone and its combination with IL-23 in Th17 cells (Fig. 3, E). The latter is consistent with our previous finding in dendritic cells that PGE_2-cAMP signaling activates the p50 subunit[54] and a report that phosphorylation of p105 S933 is PKA-dependent.[55] We therefore examined the involvement of NF-κB in *Il23r* induction by using *Nfkb1*-deficient mice (p105 KO) or CTP-NBD, a NF-κB inhibitor. Interestingly, both genetic deficiency and pharmacological inhibition of NF-κB suppressed *Il23r* induction in response to db-cAMP, IL-23 and in combination (Fig. 3, F and G).

These results together suggest that the PGE_2-EP2/EP4-cAMP-PKA signaling works together with IL-23 signaling to activate STAT3, CREB1 and NF-κB for induction of *Il23r* expression in Th17 cells.

Gene signature induced by PGE_2-EP2/EP4-cAMP signaling in $CD4^+$ T cell populations primed with IL-6 and TGF-β1

Since pathogenic Th17 cells should express various molecules in addition to IL-23R to exert their pathogenicity, we next examined how PGE_2-EP2/EP4-cAMP signaling contributes to expression of such pathogenic genes in Th17 cells. $CD4^+$ T cells were cultured under the Th17-skewing conditions with IL-6 and TGF-β1 for 3 days, then incubated with db-cAMP alone, IL-23 alone or both for 24 h, and subjected to microarray analysis. The numbers of genes up/down-regulated more than 2-folds by each stimulation were examined by Venn-diagrams (Fig. 4, A), and the genes expressed in each cluster (Table E, 1-8) were subjected to heat-map analysis (Fig E3, A) and gene ontology analysis (Fig E3, B; Table E, 9-11). Expression of representative genes in each

cluster is shown in the heat-map (Fig. 4, B). Cluster 1U included genes (e.g. *Il17a*, *Il17f*, *Il1r1* and *Il23r*) that were up-regulated by db-cAMP, IL-23, or both in combination (Fig. 4 B, left). Cluster 2U included genes (e.g. *Il22*) that were increased by IL-23 alone or its combination with db-cAMP (Fig. 4 B, left). Cluster 3U encompasses various genes, which up-regulated by db-cAMP alone or its combination with IL-23 but not IL-23 alone. They include genes involved in cell migration and adhesion such as *Ccr2, Cxcr4, Cx3cr1, Ccr6, S1pr1, Sema4f, Sema6c, Efna2, Sell, Selp* and *Itgb3*, those involved in induction of IFN-γ, such as *Il12rb2, Il18r1* and *Il18rap*, and those involved in cell activation such as *Tlr4, Tgfb3, Rasa, Rasgrp2, Lat2, Txk* and *Rora* (Fig. 4 B, left). Cluster 4U include genes such as *Il1b, Il17rc, Il17re, Prkcq, Sema3c, Sema6a* and *Tlr12* that are up-regulated by the combination of IL-23 and db-cAMP only (Fig. 4 B, left). On the other hand, the genes in Cluster 3D and Cluster 4D were down-regulated by db-cAMP, and contained *Il10, Il2, Il4, Il5, Il13* and *Il9*, which are known as suppressive factors of inflammation, (Fig. 4 B, right). Expression of the representative genes was then confirmed by qRT-PCR analysis. Expression of *Il17a*, *Il17f* and *Il23r* in Cluster 1U, *Il18r1, Il18rap, S1pr1, Ccr2, Cxcr4, Tlr4, Cxcl3, Cx3cr1, Sema4f, Sell* and *Txk* in Cluster 3U and *Il17re, Sema3c* and *Sema6a* in Cluster 4U was all up-regulated (Fig. 4, C), and expression of *Il10* in Cluster 3D was down-regulated by the addition of db-cAMP compared to incubation with IL-23 alone (Fig. 4, D). Thus, signaling through cAMP regulates expression of various genes that are not regulated by IL-23 alone, and may confer pathogenic property to Th17 cells.

T cell-intrinsic PGE_2-EP2/EP4 signaling is critical in IL-23-mediated psoriatic skin inflammation *in vivo*

Accumulating evidences suggest that Th17 cells become pathogenic via the IL-23-IL23 receptor axis and play crucial roles in development of various autoimmune diseases including psoriasis.[8,56,57] However, how these Th17 cells acquired the pathogenicity *in vivo* and to what extent the microenvironment of diseases contributes to this process remain to be defined. In the IL-23-induced psoriasis mouse model, gene expression of enzymes involved in PGE_2 biosynthesis including *Ptgs2* encoding COX-2, *Ptges* encoding membrane-associated PGE synthase, mPGES1, and *Ptges2* encoding membrane-associated PGE synthase-2, mPGES2, were all up-regulated by IL-23 administration into the skin (Fig E4, A), which is consistent with clinical observation that local PGE_2 levels are elevated in blister fluids from human psoriatic skin.[58] We therefore hypothesized that IL-23 possibly activates PGE_2–EP2/EP4 signaling, which may contribute to psoriasis pathogenesis.

To test this hypothesis, we injected IL-23 into the skin of WT C57BL/6N mice as well as EP2 knockout (KO) mice[46] with or without administration with a EP4 antagonist, AS1954813,[49] and assessed skin inflammation by ear thickness and histology. The EP2 deficiency or the EP4 antagonism alone reduced IL-23-induced ear swelling by half and attenuated edema and cell infiltration, and, when combined, led to nearly complete suppression of IL-23-dependent skin inflammation (Fig. 5, A and B). Blockade of EP2 and/or EP4 caused no alteration in PBS-injected control ear (Fig E3, B). To examine at which step of inflammation EP2 deficiency and EP4 antagonism exert their effects and whether it is related to generation of pathogenic Th17 cells, we digested ear tissues and analyzed $CD4^+$ T cell populations in the skin by flow cytometry. While there were little numbers of cells producing IL-17A or IFN-γ in PBS-injected control ear, significant accumulation of the $IL\text{-}17A^+$ and $IL\text{-}17A^+IFN\text{-}\gamma^+$ $CD4^+$ T cell

352 populations were observed in the IL-23-injected ear as observed in psoriasis dermis in
353 psoriasis patients.[59] The IL-17A$^+$IFN-γ^+ CD4$^+$ T cell population is suggested one
354 population of pathogenic Th17 cells.[60] This CD4$^+$ T cell population was shown to arise
355 in an IL-23-dependent manner from adoptively transferred T cells in transfer colitis,[26]
356 and may reflect the Th17 to Th1 reprograming at inflammatory sites as shown for
357 antigen-specific Th17 cells transferred to NOD mice.[22] This accumulation was
358 significantly reduced by blockade of either EP2 or EP4 alone and nearly completely
359 suppressed by blockade of both EP2 and EP4 (Fig. 5, C and Fig E4, C-E). Consistently,
360 expression of *Il17a* and *Ifng* that was up-regulated in the IL-23-injected ear was also
361 reduced to the negligible levels by combined EP2 and EP4 blockade (Fig. 5 D, left and
362 middle). Notably, EP2 and EP4 blockade also markedly inhibited enhanced expression
363 of *Il23r* by IL-23 injection (Fig. 5 D, right). These findings together indicate that the
364 EP2/EP4 signaling is indeed involved in the generation of pathogenic Th17 cells and
365 elicitation of inflammation in this model. We then asked whether T-cell intrinsic
366 EP2/EP4 signaling is responsible for these IL-23-induced phenotypes. To this end, we
367 used EP2$^{flox/flox}$ mice[47] and EP4$^{flox/flox}$ mice[48] and generated EP2$^{flox/flox}$ EP4$^{flox/flox}$ Lck-
368 Cre$^+$ mice. EP2$^{flox/flox}$ EP4$^{flox/flox}$ Lck-Cre$^+$ mice showed no significant differences in the
369 numbers of total cells, B cells, T cells, CD4 T cells, CD8 T cells, Th1 cells, Th17 cells
370 and Treg cells in the thymus, spleen, lymph node and peripheral blood compared to
371 control WT Lck-Cre$^+$ mice (Fig E5, A). However, deficiency of both EP2 and EP4
372 selectively in T cells prevented accumulation of Th17 cells in the ear and almost
373 completely attenuated IL-23-induced skin inflammation (Fig. 5, E and F). These results
374 together therefore suggest that the T cell-intrinsic PGE_2-EP2/EP4 signaling is critical
375 for the generation of pathogenic Th17 cells in psoriasis model. We also performed

imiquimod (IMQ)-induced psoriasis model[8], in which we applied IMQ to the ear of WT or EP2 KO mice with or without EP4 antagonist for 6 days (Fig E6, A). We found that ear swelling was also significantly reduced by EP2 deficiency and EP4 antagonism and additively in combination similar to the results in IL-23-induced psoriasis model.

Given the above findings, we next examined the effects of COX inhibitors on skin inflammation in IL-23-induced psoriasis model (Fig E6, B and C). Treatment with indomethacin and SC-236 significantly suppressed the IL-23-induced ear swelling with concomitant suppression of IL-17A$^+$ and IL-17A$^+$ IFN-γ^+ cells in the skin (Fig E6, B and C). These findings together suggest that COX inhibitors are as potent as EP2 and EP4 antagonists in suppressing skin inflammation at least in this model.

PGE_2 signaling positively correlates with the IL-23/Th17 pathway in human psoriatic skin biopsies

Finally, to extrapolate our findings in mice to humans, we analyzed a public microarray dataset on gene expression profiles in skin biopsies from psoriasis patients and healthy control individuals,[61] with a particular interest in correlation of PGE_2 signaling and the IL-23/Th17 pathway. As expected, psoriatic lesional skin overexpressed Th17 signature genes (including *IL23A*, *IL12B*, *IL23R*, *IL17A*, *IL17F*, and *IL22*), *STAT3* and *NFKB1* (encoding NF-κB p105) (Fig. 6, A). Moreover, psoriatic lesional skin overexpressed enzymes in PGE_2 biosynthesis, e.g., *PTGS2*, *PTGES* and *PTGES2*, and the EP4 receptor (*PTGER4*) but under-expressed the PGE_2 degrading enzyme, 15-PGDH (encoded by *HPGD*) (Fig. 6, A). Interestingly, expression of Th17 signature genes positively correlated with those involved in PGE_2 biosynthesis (e.g. *PTGES* and *PTGES2*) and receptor (e.g. *PTGER4*) but negatively correlated with *HPGD* (Fig. 6, B). In addition,

400 the clinically effective anti-IL-23 therapy[62] down-regulated gene expression of not only
401 the IL-23/IL-17 pathway (e.g., *IL23A*, *IL23R*, *IL17A*) but also those in PGE synthesis
402 like *PTGES* (Fig. 6, C and D). These findings support a potential crosstalk between the
403 PGE_2 and IL-23/IL-17 pathways also in human psoriatic skin inflammation.

DISCUSSION

The IL-23-IL-23 receptor signaling plays a critical role in generation of pathogenic Th17 cells in autoimmunity.[5-9] However, there remain several issues to be solved on this action, namely how this signaling gets promoted, what transcriptional mechanisms other than STAT3 are involved, what, along with the IL-23 signaling, makes Th17 cells pathogenic, whether and how much such mechanism operates *in vivo* and how relevant are the findings obtained in mouse to humans. Given the previously reported action of PGE_2 on Th17 expansion,[43-45] we focused here on PGE_2 action in Th17 pathogenicity to address these issues.

We have first found that PGE_2 synergizes with IL-23 and enhances *Il23r* expression through EP2 and EP4, a finding consistent with the findings in human Th17 cells.[44] We have then found that IL-23 stimulation induces PGE_2 production in Th17 cells and the IL-23-induced *Il23r* expression was attenuated by the treatment of cells with indomethacin or EP2/EP4 antagonists. These results thus suggest a previously unsuspected intrinsic amplification mechanism mediated by the PGE_2-EP2/EP4 signaling in Th17 cells that helps trigger the initial IL-23 responses in premature Th17 cells.

We have further analyzed the transcriptional mechanisms underlying the synergistic action of IL-23 and PGE_2, and found that this action is mediated by not only STAT3 but also CREB1 and NF-κB. The involvement of CREB1 is analogous to that in the PGE_2-EP2/EP4-mediated *Il12rb2* induction during Th1 cell differentiation,[42] and may be consistent with the findings by Hernandez *et al.*[63] showing that the CREB1/CRTC2 pathway regulates expression of IL-17A and IL-17F and that Th17 differentiation is defective in CRTC2 mutant mice. IL-23R and IL-12Rβ2 make a pair

with the same molecule, IL-12Rβ1, to form IL-23 receptor and IL-12 receptor, respectively. It is interesting that the same pathway regulates expression of these two genes. We have also used T cells from p105 NF-κB1-deficeint mice and CTP-NBD and unraveled the involvement of NF-κB in the IL-23/cAMP-induced *Il23r* expression in Th17 cells. Consistent with these findings, we previously found that PGE_2 through EP2 or EP4 activates NF-κB1-containing NF-κB in various types of cells including macrophages and dendritic cells, and induces expression of inflammation-related genes including COX-2, which then produces PGE_2 and amplifies this process.[47,54,64] Our present findings thus further extend the importance of this COX-2-PGE_2-EP2/EP4-NF-κB loop to generation of Th17 cell pathogenicity. On the other hand, Boniface *et al*. suggested that PGE_2-induced enhancement of *Il23R* expression in human Th17 cells was mediated by IL-1β-IL-1 receptor pathway.[44] This is also a possibility in mice, because up-regulated expression of *Il1r1* and *Il1b* was detected in Cluster 1U and Cluster 4U by our microarray analysis (Fig. 4 B, left). However, we assume that this mechanism does not critically operate in our experiment, because the addition of anti-IL-1β antibody to the medium did not reduce the *Il23r* induction (Fig E7).

In addition to *Il23r,* our microarray analysis has revealed that stimulation of the EP2/EP4 signaling together with IL-23 facilitates expression of a variety of pathogenic Th17 signature genes (i.e. *Il17a*, *Il17f*, *Il18r1* and *Tgfb3*). Interestingly, PGE_2-EP2/EP4 signaling also up-regulated the expression of various genes related to chemotaxis and migration such as *S1pr1*, *Ccr2*, *Cxcl3*, *Cx3cr1*, *Cxcr4*, *Sema4f*, *Sell*, *Sema3c* and *Sema6a* (Fig. 4 B, left). These results suggest that PGE_2-EP2/EP4 signaling may contribute to migration, infiltration and accumulation of Th17 cells into inflammation

lesion. On the other hand, the addition of db-cAMP down-regulated expression of *Il10*, *Il2*, *Il4* and *Il9*, which are known as suppressive factors for Th17 cells. Although some of these results such as IL-17A are consistent with the previous findings in human Th17 cells,[44] our study did not detect induction of IFN-γ and T-bet in cultured Th17 cells, which may reflect the stages of Th17 cells examined in each study.[20,24,65] It should also be noted that our analysis was carried out in the whole $CD4^+$ T cell population pretreated with IL-6 and TGF-β1 and stimulated with each stimulus, in which IL-$17A^+$ cells comprise about 10%. Single cell RNA sequencing analysis is therefore desired to establish the gene expression signatures specific to Th17 cells matured with each stimulus.

Nonetheless, the most important point in our study was that the EP2/EP4 signaling in Th17 cells identified here is critical in eliciting their pathogenicity in vivo in immune inflammation. We tested this issue in IL-23-induced mouse psoriasis model. Intriguingly, not only the systemic inhibition of EP2/EP4 signaling using the EP4 antagonist in EP2 KO mice but also selective loss of EP2 and EP4 in T cells almost completely suppressed inflammation induced by IL-23. This was accompanied by suppression of accumulation of IL-$17A^+$ and IL-$17A^+$IFN-γ^+ T cells and suppression of expression of *Il17a*, *Ifng*, *Il23r* genes in the lesion. These results suggest that the PGE_2-EP2/EP4 signaling functions critically in generation of pathogenic Th17 cells induced by IL-23 *in situ*. Of those Th17 cells, antigen-specific Th17 cells were shown to be specifically involved in the pathogenesis of mouse models of autoimmune inflammation including EAE,[66] type 1 diabetes,[22] and psoriasis.[67] Quite recently it was also reported that mPGES1 is involved in generation of antigen-specific Th17 cells by regulating PGE_2 production in a T cell-autocrine and paracrine manner.[68] Our present findings

combined together with these findings suggest that PGE_2 plays an important role in psoriasis through the regulation of antigen-specific pathogenic Th17 cells.

The present study also showed that EP2 deficiency and EP4 antagonism also significantly suppressed the psoriatic inflammation in IMQ model. Notably, however, the combined EP2 deficiency and EP4 antagonism did not completely suppress ear swelling in this model, possibly because there is the IL-17-independent component in skin inflammation in this model.[8]

In this study, we also tested the effect of COX inhibitors in IL-23-induced psoriasis model, and found that COX inhibitors are as potent as EP2 and EP4 antagonist in suppressing psoriasis-like skin inflammation in this model. The question is whether COX inhibitors are beneficial in Th17-driven human autoimmune diseases. COX inhibitors, particularly Celecoxib, are used for treatment of the early stage of rheumatoid arthritis patients, and in patients with mild psoriatic arthritis.[69] In these cases, COX inhibitors produce good symptomatic relief. While this effect is ascribed to their analgesic and general anti-inflammatory actions, our study suggests that it may be derived at least in part from their suppressive action on Th17-mediated pathology, a possibility that should be tested in future. On the other hand, COX inhibitors have less appreciable therapeutic benefits in established psoriasis and advanced RA in human patients. There are several plausible reasons. PG-mediated process may be critical in triggering pathogenic Th17 cell generation, but not so in advanced stage of diseases that might be dominantly regulated by established Th17 cells. Another may be due to the fact that PGs cause immune inflammation not by acting alone but by working with cytokines and boosting and modifying their actions. COX inhibitors may therefore exert therapeutic benefits more effectively when combined with anti-cytokine drugs, and

lessen the dose of the latter. Finally, COX inhibitors may divert arachidonate metabolism to leukotriene. Recent studies suggest that leukotrienes facilitate maturation and migration of Th17 cells.[70,71] Further studies need to be conducted to unravel these issues.

Another topic to be discussed on PGE_2 in psoriasis is its facilitative action in ultraviolet (UV) irradiation therapy, which at a glance contradicts our present findings on the facilitative action of PGE_2 on Th17 pathogenicity. UVB-irradiation is an effective therapeutic treatment of psoriasis by inducing immunosuppression.[72] We previously showed that UVB induces PGE_2 in the epidermis and PGE_2-EP4 signaling mediates systemic immunosuppression via the up-regulation of RANKL in keratinocytes and inducing regulatory T cells.[73] Thus, the PGE_2-EP4 signaling in this case facilitates immunosuppression and not immune activation. One point is that UVB does not penetrate to the dermis and the events it causes are within the epidermis, while IL-23-induced inflammatory events occur in the dermis. Another point is difference in the context, UVB irradiation in the UV therapy and IL-23 in psoriatic inflammation. PGE_2 alone does not induce either effects, but functions directionally dependent on the context.

Finally, we have examined the relevance of our findings to human diseases by analyzing biopsy samples from psoriasis patients. Psoriatic lesional skin over-expressed not only Th17 signature genes including *IL23A*, *IL12B*, *IL23R*, *IL17A*, *IL17F*, *IL22*, *STAT3* and *NFKB1*, but also those involved in PGE_2 biosynthesis and function such as *PTGS2*, *PTGES*, *PTGES2* and *PTGER4*. Expression of Th17 signature genes shows positive correlation with *PTGES*, *PTGES2* and *PTGER4*, and negative correlation with *HPGD*, and the anti-IL-23 therapy down-regulated expression of not only genes in the

IL-23/IL-17 pathway (e.g., *IL23A*, *IL23R*, *IL17A*) but also those in PGE_2 synthesis, suggesting that these two are functionally linked. These findings together with the finding by Kofler *et al.* that EP2 is expressed in Th17 cells from multiple sclerosis patients and that forced expression of EP2 in healthy Th17 cells triggers expression of pathogenic genes[74] indicate that T cell-intrinsic EP2/EP4 signaling is critical in IL-23-driven Th17 cell pathogenesis also in humans, and support a view that the combined inhibition of EP2 and EP4 is of value in therapeutic intervention of various Th17-mediated diseases.

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859 **FIGURE LEGENDS**

860 Figure 1. **IL-23 mobilizes the endogenous PGE_2-EP2/EP4-cAMP-PKA pathway to**
861 **facilitate Th17 expansion through synergistic *Il23r* induction. (**A and B**)** Expansion
862 of Th17 population by PGE_2 and IL-23. $CD4^+$ T cells were differentiated with TGF-β1
863 and IL-6 to Th17 cells for 4 days, then stimulated with 100 nM PGE_2 in the absence or
864 presence of IL-23 (10 ng/ml) for additional 3 days. The cells were examined by FACS
865 for IL-17A and IFN-γ (A) and by qRT-PCR for *Il17a* expression (B). **(**C-E**)** Effects of
866 PGE_2, agonists selective to each EP subtype and related compounds on *Il23r* expression.
867 Th17 cells were incubated with 100 nM PGE_2, an agonist selective to each EP subtype,
868 ONO-DI-004 (EP1), ONO-AE1-259 (EP2), ONO-AE-248 (EP3), or ONO-AE1-329
869 (EP4), 100 μM dibutyryl cAMP (db-cAMP), 10 μM forskolin (FSK), 100 μM 3-
870 isobutyl-1-methylxanthine (IBMX) with or without IL-23. *Il23r* expression (C and D)
871 or IL-17A concentrations in culture supernatant (E) was examined. Expression of *Il23r*
872 in Th17 cells stimulated with 100 μM db-cAMP, 300 μM N6-Bnz-cAMP (a PKA
873 agonist), 300 μM 8-pCTP-2'-O-Me-cAMP (an Epac activator) (F), or H-89 (a PKA
874 inhibitor) (G) with or without IL-23. All bars indicate mean ± SEM (n=3, each in A-G).
875 $p<0.05$, **, $p<0.01$, ***, $p<0.001$.

876

877 Figure 2. **IL-23 self-amplifies its own signaling through a T cell intrinsic positive**
878 **feedback COX-2-PGE_2-cAMP-IL-23R loop.** (A) Expression of COX-2 (*Ptgs2*)
879 mRNA (A) in Th17 cells, or Th17 cells further cultured in the presence or absence of
880 IL-23 for 3 days determined by qRT-PCR. (B) Concentrations of PGE_2 in the culture
881 supernatants of Th17 cells in the presence or absence of IL-23 and indomethacin
882 determined by ELISA. n.d., not detected. (C) *Il23r* expression in Th17 cells stimulated

with PGE_2 and IL-23 in the presence or absence of indomethacin for 3 days. (D) *Il23r* expression in Th17 cells stimulated with PGE_2 and IL-23 in the presence or absence of EP2 (PF-04418948) and/or EP4 (ONO-AE3-208) antagonists for 3 days. All bars indicate mean ± SEM (n=3, each in A-D). *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$.

Figure 3. **STAT3, CREB1 and NF-κB mediate cAMP and IL-23 induced *Il23r* expression in Th17 cells.** (A) Effect of STAT3 inhibitor VII on *Il23r* expression in Th17 cells stimulated with db-cAMP and/or IL-23 for 3 days. (B) Western blot analysis of phospho-Y705 STAT3 and α-Tubulin as a loading control in Th17 cells cultured as described in Supplementary Methods. Representative images from 2 independent experiments are shown. (C) Effect of KG-501 on *Il23r* expression in Th17 stimulated by db-cAMP and/or IL-23. (D) Effects of RNA interference for CREB1 on *Il23r* expression (left) and *Creb1* expression to confirm CREB knockdown efficiency (right). RNA interference, subsequent culture and stimulation of Th17 cells were performed as described in Supplementary Methods. (E) Western blot analysis of phospho-S536 NF-κB p65 (pp65), phospho-S933 NF-κB p105 (pp105), p65, p105/p50 and α-Tubulin in Th17 cells stimulated as described in Supplementary Methods. Representative images from 2 independent experiments are shown. (F and G) Effects of p105 KO (F) or CTP-NBD (G) on *Il23r* expression in Th17 cells stimulated with db-cAMP and/or IL-23 for 3 days. All bars indicate mean ± SEM. (n=3, each in A, C, F, and G, n=18 in B) *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$.

Figure 4. **Activation of the COX-2-PGE_2-EP2/EP4-cAMP pathway confers pathogenic Th17 phenotype.** (A) Microarray analysis of gene expression profiles in

907 Th17 cells stimulated with db-cAMP and/or IL-23. Venn-diagram analysis of two folds
908 up- or down-regulated genes compared to the vehicle control upon each stimulus (One-
909 way ANOVA $p<0.05$, n=3) (left and right, respectively). (B) Heat-map analysis of
910 expression of selected genes from each cluster. (C) qRT-PCR analysis of expression of
911 representative genes of Th17 signature and immune activation in response to db-cAMP,
912 IL-23 or db-cAMP and IL-23 in combination. (D) qRT-PCR analysis of expression of a
913 representative inflammation suppressor gene, *Il10*, in response to db-cAMP, IL-23 or
914 db-cAMP and IL-23 in combination. All bars in C and D indicate mean ± SEM. (n=3) *,
915 $p<0.05$, **, $p<0.01$, ***, $p<0.001$.
916
917 Figure 5. **PGE_2-EP2/EP4 signaling in T cells is required for IL-23-driven psoriatic**
918 **skin inflammation.** (A-D) Ear swelling (A) (n=16-17), representative Hematoxylin-
919 Eosin (HE) staining of the histological section of the ear (B) (n=3-4), number of IL-
920 $17A^+$ and IL-$17A^+$ IFN-γ^+ $CD4^+$ T cells of the ear (C) and gene expression of *Il17a*, *Ifng*
921 and *Il23r* in the whole ear tissue (D) of WT or EP2 KO mice subcutaneously injected
922 with IL-23 or PBS into the ear daily. An EP4 antagonist (AS1954813, 100 mg/kg) or
923 vehicle was orally administered twice a day to the indicated mice. Bars in (B), 50 μm.
924 Representative quantification results of the cell number in each population from 4
925 independent FACS experiments are shown in (C) (n=3). Gene expression was indicated
926 as fold-change compared to PBS-injected ear in (D) (n=3). (E and F)
927 $EP2^{flox/flox}EP4^{flox/flox}$Lck-$Cre^+$ mice and control WT Lck-Cre^+ mice were subjected to IL-
928 23-induced psoriasis model and the ear swelling (E) (n=11 and 7, respectively) and the
929 number of IL-$17A^+$ and IL-$17A^+$ IFN-γ^+ $CD4^+$ T cells in the ear (F) (n=7 and 3,
930 respectively) were analyzed. All bars indicate mean ± SEM. *, $p<0.05$, **, $p<0.01$, ***,

$p<0.001$.

Figure 6. **PGE_2 signaling positively correlates with the IL-23/Th17 pathway in human psoriatic skin biopsies.** (A) Expression profiles of genes related to PGE_2 signaling and Th17 signature genes in human non-lesional (NL) or lesional (PL) skin biopsies from patients with psoriasis (n=58) and skin samples from healthy controls (HC, n=64). The z-score transformed values of microarray gene expression dataset GSE13355 were used. Th17 score was generated based on the average expression level of *IL23A, IL12B, IL23R, IL17A, IL17F* and *IL22* genes. (B) Correlations of *PTGES, PTGES2, HPGD* and *PTGER4* gene expression versus that of the Th17 score. Black, green and red dots indicate healthy control, non-lesional and lesional psoriatic biopsies, respectively. (C) Expression profiles of genes related to PGE_2 synthases and Th17 signature genes in human lesional skin biopsies from patients with moderate-to-severe psoriasis before (Baseline, n=22) or 12 weeks after treatment with an IL-23-specific mAb, Guselkumab, (Guselkumab, n=8). The z-score transformed values of microarray gene expression dataset GSE51440 were used. (D) Correlations of gene expression of *PTGS2* and *PTGES* versus that of *IL23R*. P values were calculated by nonparametric Wilcoxon-Mann-Whitney tests (A, C) or nonparametric Spearman correlation test (B, D).

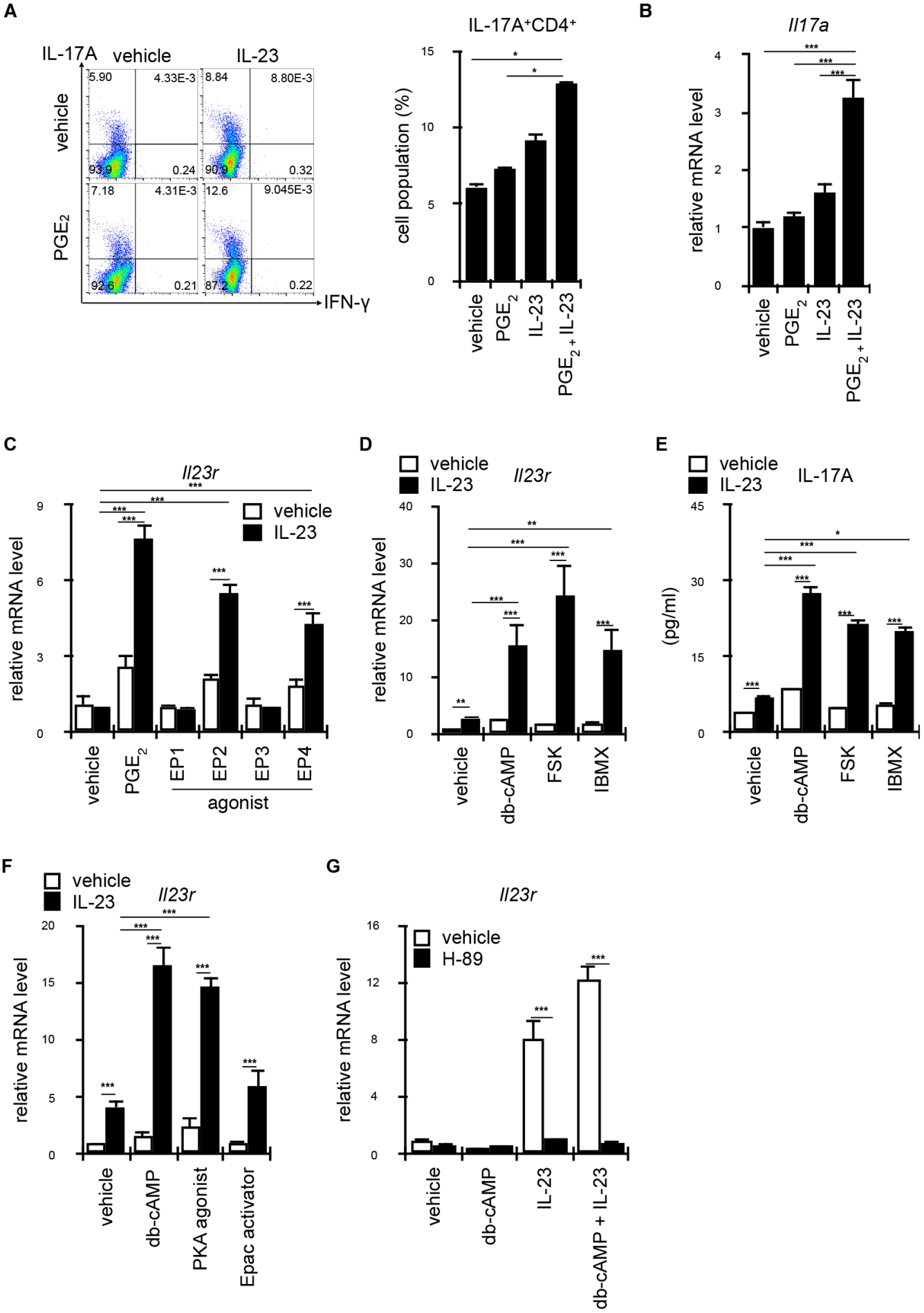
A
IL-17A
vehicle
IL-23
vehicle
PGE2
5.90
4.33E-3
8.84
8.80E-3
93.9
0.24
90.9
0.32
7.18
4.31E-3
12.6
9.045E-3
92.6
0.21
87.2
0.22
IFN-γ
IL-17A+CD4+
cell population (%)
vehicle
PGE2
IL-23
PGE2 + IL-23
B
Il17a
relative mRNA level
C
Il23r
relative mRNA level
vehicle
IL-23
EP1
EP2
EP3
EP4
agonist
D
Il23r
db-cAMP
FSK
IBMX
E
IL-17A
(pg/ml)
F
Il23r
PKA agonist
Epac activator
G
H-89
db-cAMP + IL-23

Figure. 1

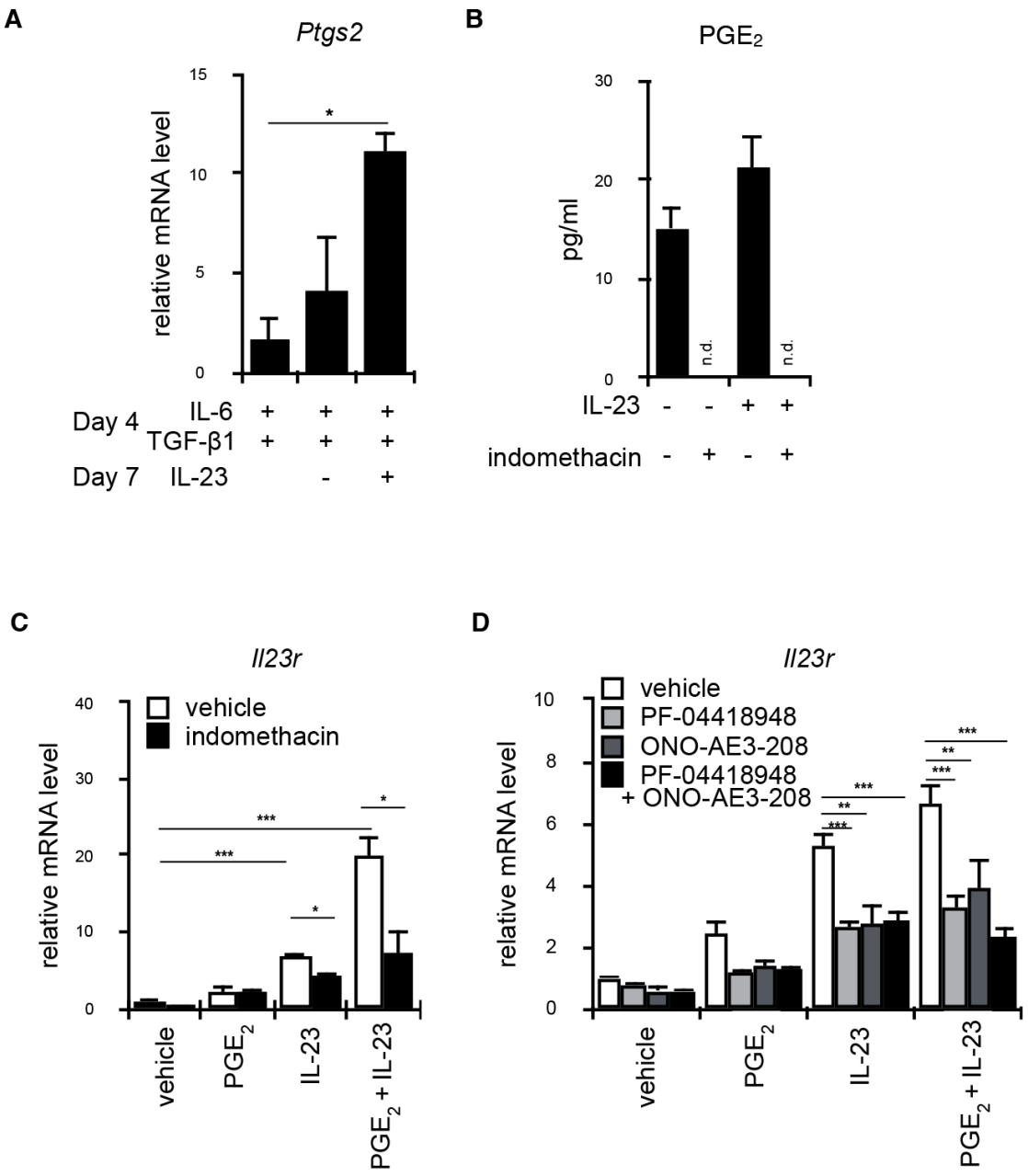
A
Ptgs2
relative mRNA level
15
10
5
0
*
Day 4
IL-6 + + +
TGF-β1 + + +
Day 7
IL-23 - +
B
PGE2
pg/ml
30
20
10
0
n.d.
n.d.
IL-23 - - + +
indomethacin - + - +
C
Il23r
relative mRNA level
40
30
20
10
0
vehicle
indomethacin
***
***
*
*
vehicle
PGE2
IL-23
PGE2 + IL-23
D
Il23r
relative mRNA level
10
8
6
4
2
0
vehicle
PF-04418948
ONO-AE3-208
PF-04418948
+ ONO-AE3-208
***
**
***
***
**
***
vehicle
PGE2
IL-23
PGE2 + IL-23

Figure. 2

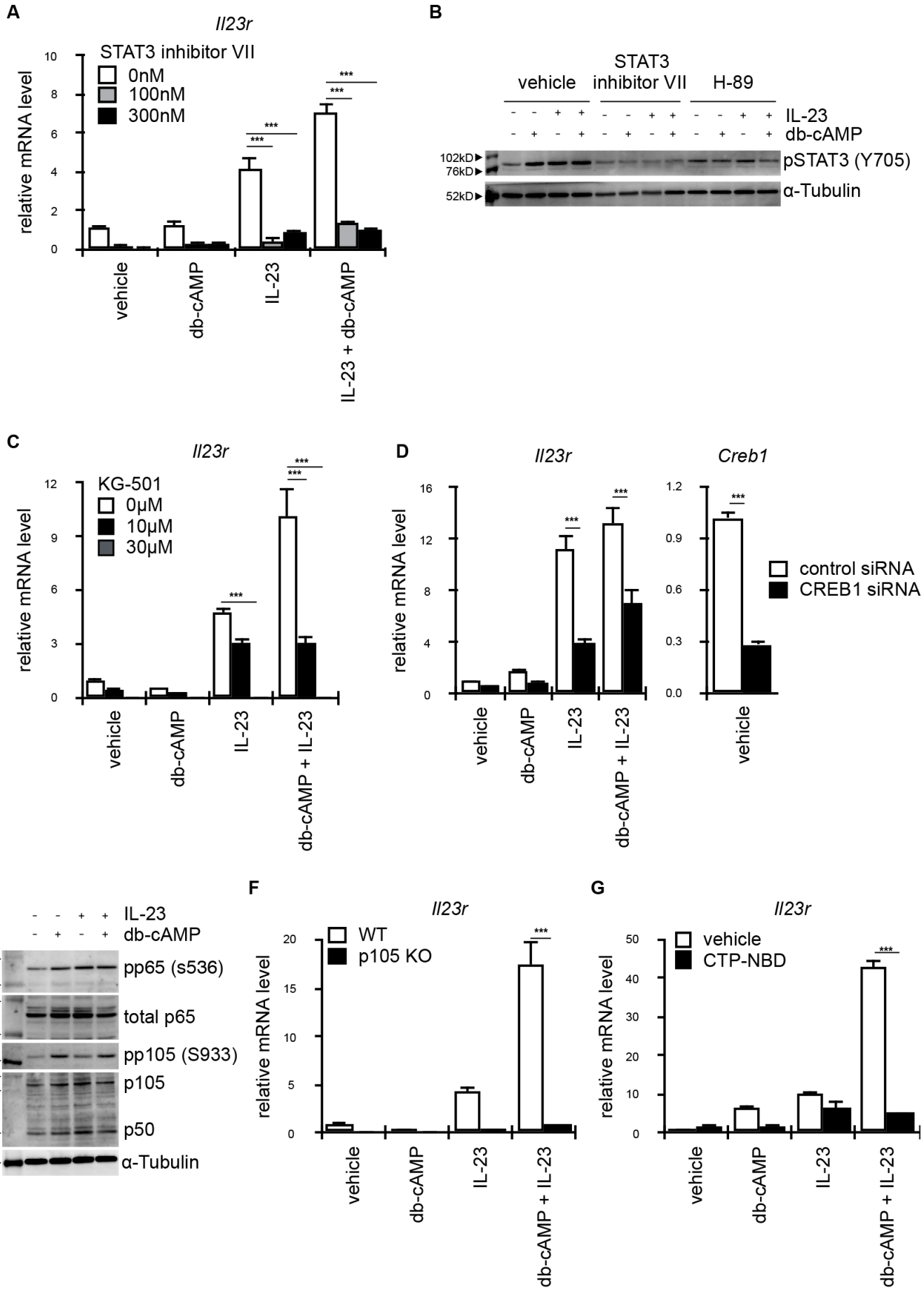
A
Il23r
STAT3 inhibitor VII
0nM
100nM
300nM
relative mRNA level
vehicle
db-cAMP
IL-23
IL-23 + db-cAMP
B
STAT3
vehicle
inhibitor VII
H-89
IL-23
db-cAMP
102kD
76kD
52kD
pSTAT3 (Y705)
α-Tubulin
C
Il23r
KG-501
0μM
10μM
30μM
relative mRNA level
vehicle
db-cAMP
IL-23
db-cAMP + IL-23
D
Il23r
Creb1
relative mRNA level
control siRNA
CREB1 siRNA
vehicle
db-cAMP
IL-23
db-cAMP + IL-23
E
IL-23
db-cAMP
76kD
52kD
pp65 (s536)
total p65
102kD
pp105 (S933)
150kD
p105
p50
α-Tubulin
F
Il23r
WT
p105 KO
relative mRNA level
vehicle
db-cAMP
IL-23
db-cAMP + IL-23
G
Il23r
vehicle
CTP-NBD
relative mRNA level
vehicle
db-cAMP
IL-23
db-cAMP + IL-23

Figure. 3

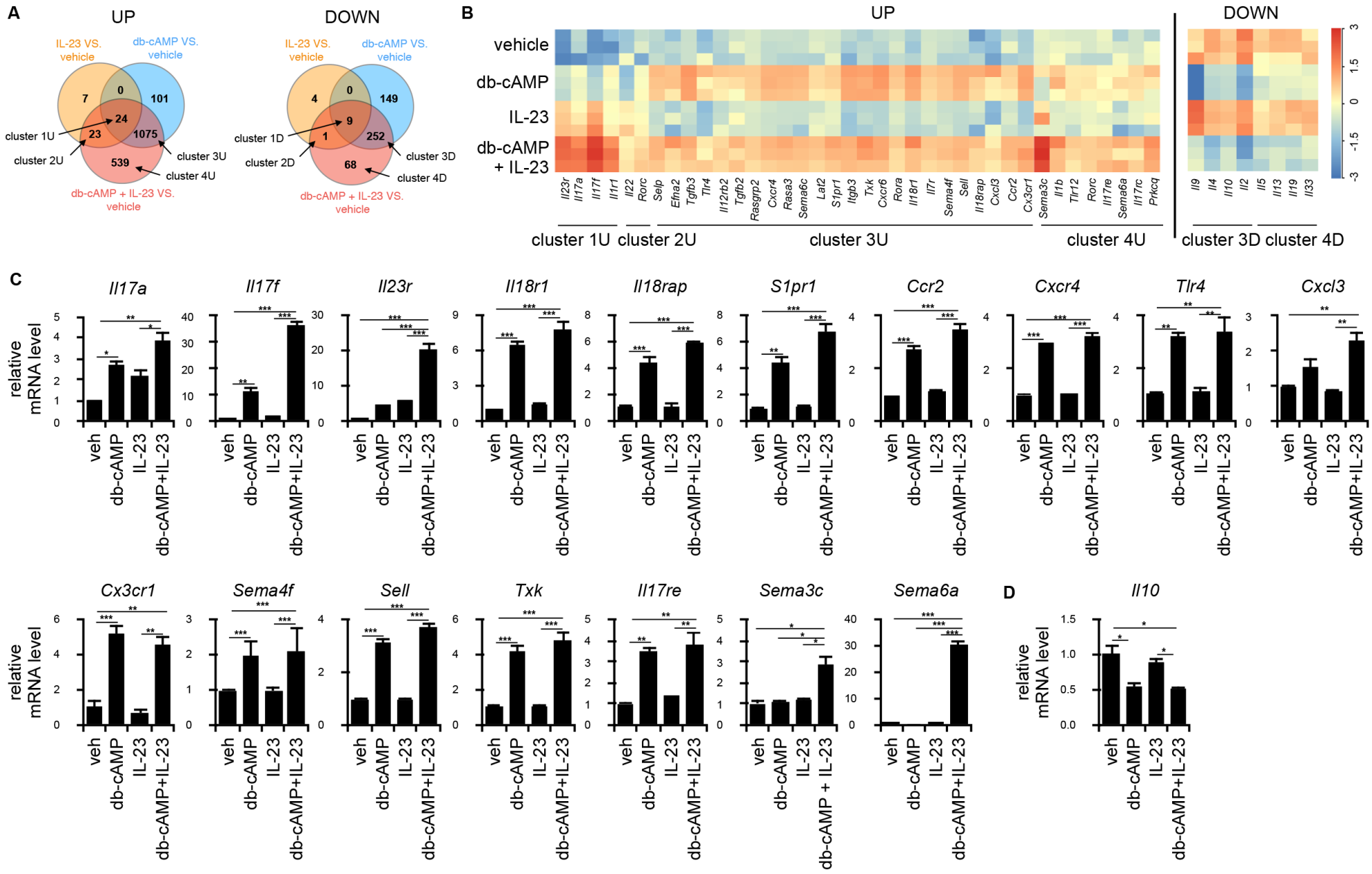
A
UP
IL-23 VS. vehicle
db-cAMP VS. vehicle
db-cAMP + IL-23 VS. vehicle
7
0
101
24
23
1075
539
cluster 1U
cluster 2U
cluster 3U
cluster 4U
DOWN
4
0
149
9
1
252
68
cluster 1D
cluster 2D
cluster 3D
cluster 4D
B
UP
DOWN
vehicle
db-cAMP
IL-23
db-cAMP + IL-23
Il23r Il17a Il17f Il1r1 Il22 Rorc Selp Efna2 Tgfb3 Tlr4 Il12rb2 Tgfb2 Rasgrp2 Cxcr4 Rasa3 Sema6c Lat2 S1pr1 Itgb3 Txk Cxcr6 Rora Il18r1 Il7r Sema4f Sell Il18rap Cxcl3 Ccr2 Cx3cr1 Sema3c Il1b Tlr12 Rorc Il17re Sema6a Il17rc Prkcq
Il9 Il4 Il10 Il2 Il5 Il13 Il19 Il33
3 1.5 0 -1.5 -3
cluster 1U cluster 2U cluster 3U cluster 4U
cluster 3D cluster 4D
C
Il17a
Il17f
Il23r
Il18r1
Il18rap
S1pr1
Ccr2
Cxcr4
Tlr4
Cxcl3
Cx3cr1
Sema4f
Sell
Txk
Il17re
Sema3c
Sema6a
relative mRNA level
veh
db-cAMP
IL-23
db-cAMP+IL-23
db-cAMP + IL-23
D
Il10
relative mRNA level

Figure. 4

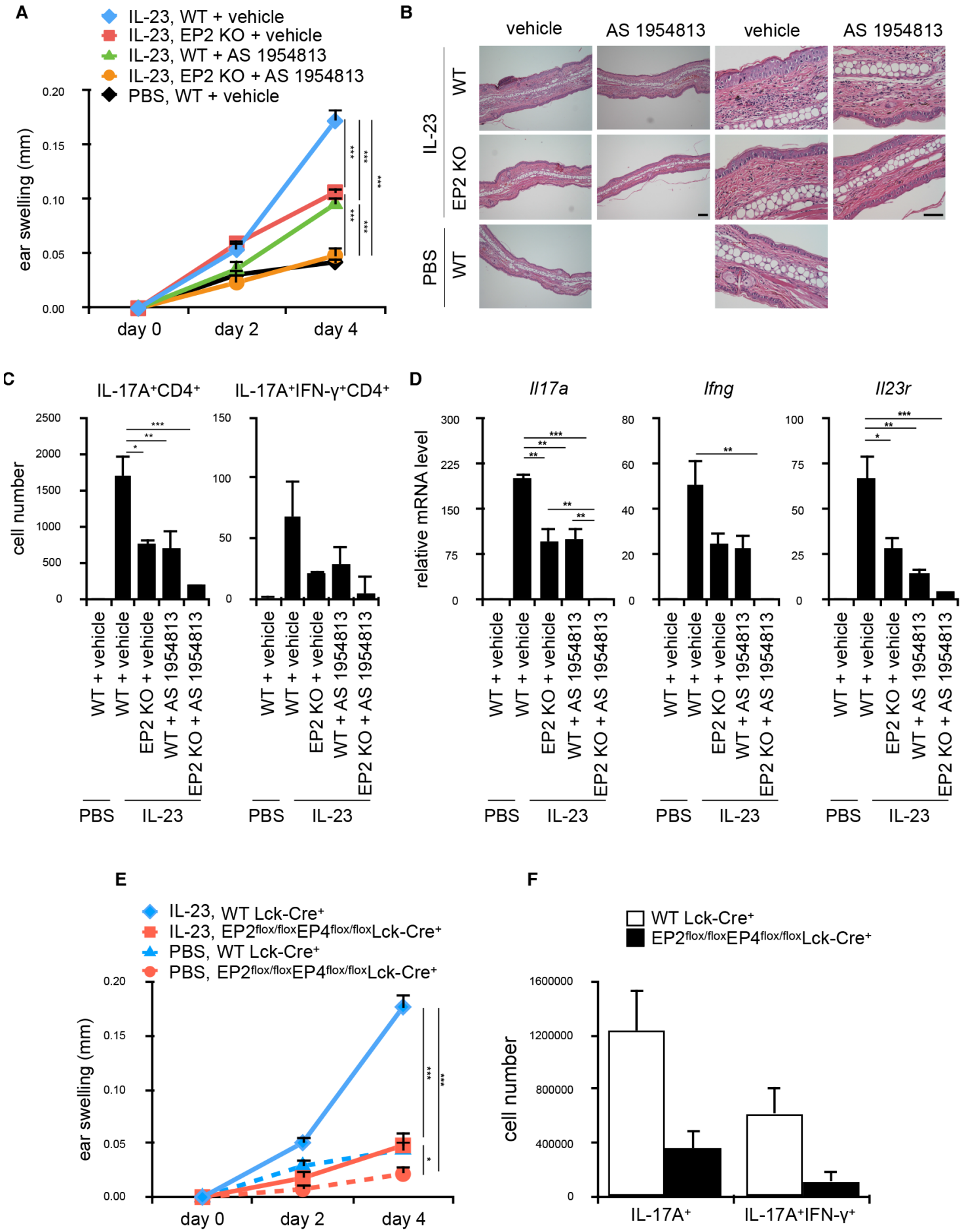
A
IL-23, WT + vehicle
IL-23, EP2 KO + vehicle
IL-23, WT + AS 1954813
IL-23, EP2 KO + AS 1954813
PBS, WT + vehicle
ear swelling (mm)
day 0
day 2
day 4
B
vehicle
AS 1954813
vehicle
AS 1954813
IL-23
WT
EP2 KO
PBS
WT
C
IL-17A⁺CD4⁺
IL-17A⁺IFN-γ⁺CD4⁺
cell number
WT + vehicle
WT + vehicle
EP2 KO + vehicle
WT + AS 1954813
EP2 KO + AS 1954813
PBS
IL-23
D
Il17a
Ifng
Il23r
relative mRNA level
E
IL-23, WT Lck-Cre⁺
IL-23, EP2flox/floxEP4flox/floxLck-Cre⁺
PBS, WT Lck-Cre⁺
PBS, EP2flox/floxEP4flox/floxLck-Cre⁺
ear swelling (mm)
day 0
day 2
day 4
F
WT Lck-Cre⁺
EP2flox/floxEP4flox/floxLck-Cre⁺
cell number
IL-17A⁺
IL-17A⁺IFN-γ⁺

Figure. 5

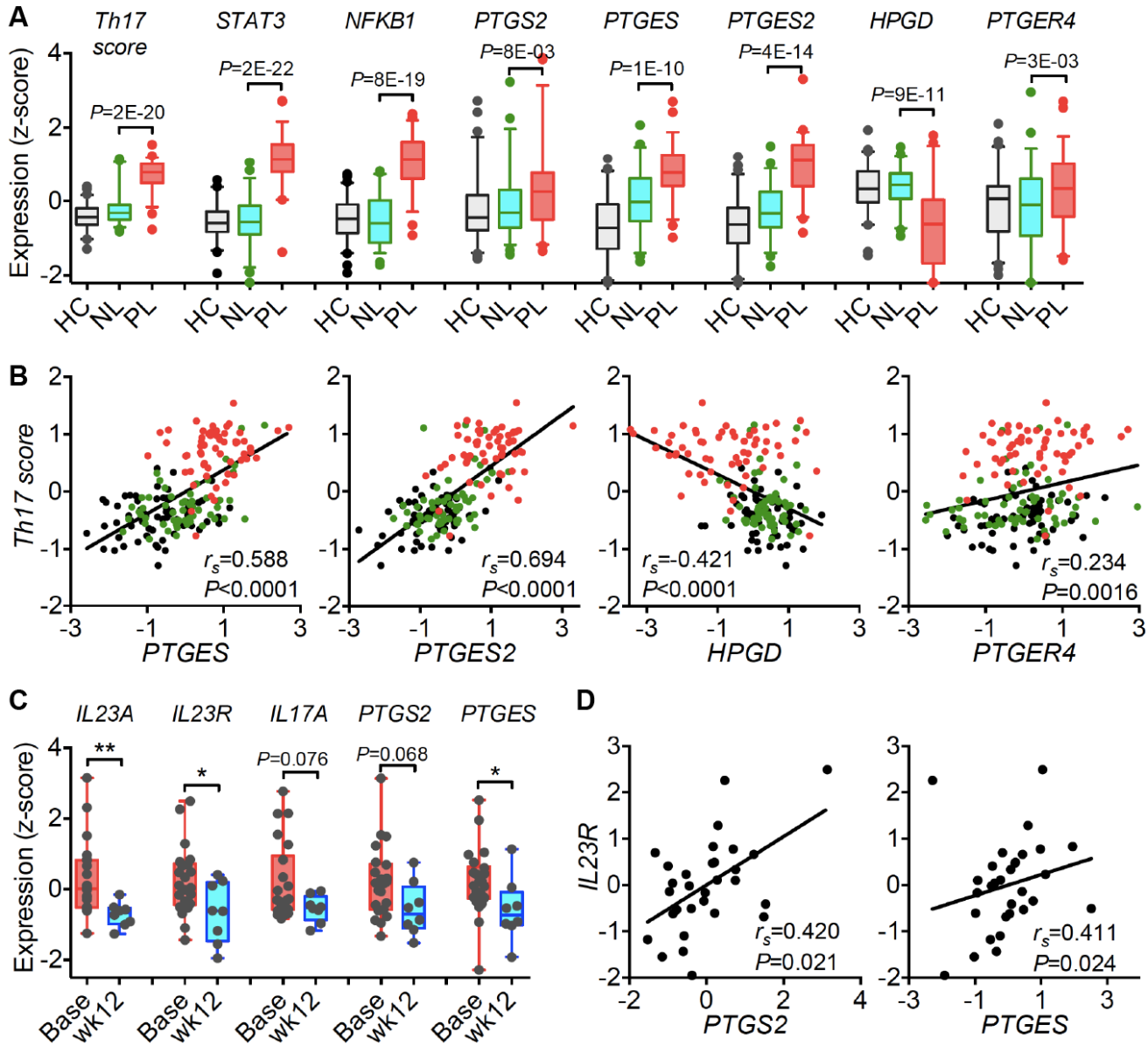
A
Th17 score
STAT3
NFKB1
PTGS2
PTGES
PTGES2
HPGD
PTGER4
P=2E-20
P=2E-22
P=8E-19
P=8E-03
P=1E-10
P=4E-14
P=9E-11
P=3E-03
Expression (z-score)
HC
NL
PL
B
Th17 score
PTGES
PTGES2
HPGD
PTGER4
rs=0.588
P<0.0001
rs=0.694
P<0.0001
rs=-0.421
P<0.0001
rs=0.234
P=0.0016
C
IL23A
IL23R
IL17A
PTGS2
PTGES
**
*
P=0.076
P=0.068
*
Expression (z-score)
Base
wk12
D
IL23R
PTGS2
PTGES
rs=0.420
P=0.021
rs=0.411
P=0.024

Figure. 6

SUPPLEMENTARY METHODS

Purification of $CD4^+$ T cells and differentiation into Th17 cells

Spleen was dissected from 6-10 week-old female C57BL/6N mice and cells were dissociated and collected. $CD4^+$ T cells were purified from spleen cells by magnetic activated-cell sorting (MACS) using anti-CD4 microbeads (L3T4) (#130-049-201, Miltenyi) on auto-MACS (Miltenyi). The purity of $CD4^+$ T cells was ~98 % (n=3) as assessed by FACS (FACS LSR Fortessa, BD Bioscience, San Jose, CA). Purified $CD4^+$ T cells were differentiated into Th17 cells by the combination of TGF-β1 (1 ng/ml, #240-B-002, R&D systems, Minneapolis, MN) and IL-6 (20 ng/ml, R&D systems) in the presence of 5 μg/ml of anti-CD3 antibody (#14-0031-86, eBioscience, San Diego, CA) and 2.5 μg/ml of anti-CD28 antibody (#14-0281-86, eBioscience, San Diego, CA) in RPMI-1640 medium containing 10 % fetal bovine serum (FBS) for 4 days. Differentiated cells were then collected, washed and again plated for experiments with TCR stimulation. Experimental condition of each experiment is shown in the **Results** or the **Figure Legends unless specified otherwise**.

Reagents

Agonists selective to each PGE_2 receptor subtype, EP1, EP2, EP3 and EP4 (ONO-DI-004, ONO-AE1-259, ONO-AE-248 and ONO-AE1-329 respectively) and an EP4 antagonist, ONO-AE3-208,[E1] were kindly provided by Ono Pharmaceutical Co., Osaka, Japan. An EP2 antagonist, PF-04418948, was synthesized according to the previous report.[E2] An EP4 antagonist, AS1954813, was kindly provided by Astellas Pharmaceutical Co. (Tsukuba, Japan). PGE_2, SC-560 and SC-236 were purchased from Cayman Chemical, Ann Arbor, MI. Dibutyryl cAMP (db-cAMP), forskolin, the N6-Bnz-cAMP, the 8-pCTP-2'-O-Me-cAMP, indomethacin and KG-501 were purchased from Sigma, St. Louis, MO. STAT3 inhibitor VII, Src Kinase Inhibitor-I, H-89, and CPT-NBD peptides were purchased from Calbiochem, San Diego, CA. Mouse IL-1β/IL-1F2 Antibody (AF-401-SP) was purchased from R&D systems.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

RNA purification and reverse transcription were performed by the RNeasy Mini Kit (Qiagen GmbH, Hilden, Germany) and the High-capacity cDNA Reverse Transcription Kit (ABI biosystems, Grand Island, NY) according to manufacturers' instructions. cDNA, primers and FastStart DNA MasterPLUS SYBR Green (Takara, Shiga, Japan) were then mixed in 96-well PCR plate, and quantitative PCR was performed using CFX96 Real-Time System (Biorad). The following primers were used in this study;

Gapdh: forward 5′-TGAACGGGAAGCTCAC-3′and reverse 5′ -TCCACCACCCTGTTGC-3′

Il17a: forward 5′-TGTGAAGGTCAACCTCAAAGTC-3′ and reverse 5′ -GAGGGATATCTATCAGGGTCTTCA-3′

Il23r: forward 5′-CCAAGTATATTGTGCATGTGAAGA-3′ and reverse 5′ -AGCTTGAGGCAAGATATTGTTGT-3′

Ptgs2: forward 5′- TCGCAGGAAGGGGATGTTGT -3' and reverse 5'- CTGAAGCCCACCCCAAACAC -3′

45 *Creb1*: forward 5′-CCAAACTAGCAGTGGGCAGT-3′ and reverse 5′

46 -CCCCATCCGTACCATTGTT-3′

47 *Il17f*: forward 5′-GGAAGACAGCACCATGAAC-3′ and reverse

48 5′-TGGACAATGGGCTTGACAG-3′

49 *Il18r1*: forward 5′-GTTGAGATGGAGGATGAGGG-3′ and reverse 5′

50 -GACAGAAAACACGCAGGAG-3′

51 *Il18rap*: forward 5′-AGCCTTTAACTCTCCCCTG-3′ and reverse 5′

52 -ACACCACCTCTTCCTTCTTC-3′

53 *S1pr1*: forward 5′-CATTCTCATCTGCTGCTTCATC-3′ and reverse 5′

54 -CCACAAACATACTCCCTTCCC-3′

55 *Ccr2*: forward 5′-TGAGAAGAAGAGGCACAGG-3′ and reverse 5′

56 -CAACAAAGGCATAAATGACAGG-3′

57 *Cxcr4*: forward 5′-ATCTGTGACCGCCTTTACCC-3′ and reverse 5′

58 -ATCCTTGCTTGATGACCCCC-3′

59 *Tlr4*: forward 5′-CTTTCACCTCTGCCTTCAC-3′ and reverse 5′

60 -TACAATTCCACCTGCTGCC-3′

61 *Cxcl3*: forward 5′-GAACACCCTCAGGCTCAAGG-3′ and reverse 5′

62 -CCACCAACCAAAGAATACACATGG-3′

63 *Cx3cr1*: forward 5′-ACAAAGAGAAAGGACAACGAG-3′ and reverse 5′

64 -TGATGCGGAAGTAGCAAAAG-3′

65 *Sema4f*: forward 5′-AAGAAAGGCAAGAAAGAGGAC-3′ and reverse 5′

66 -CACATCAATAACCCCGCAC-3′

67 *Sell*: forward 5′-TGCCAAGAGACAAACAGAAG-3′ and reverse 5′

68 -CCAGCCAAATGAGAAATGCC-3′

69 *Txk*: forward 5′-CACCGAAAGACATCTCTTCC-3′ and reverse 5′

70 -ACAACCCCAAACTGACCAC-3′

71 *Il17re*: forward 5′-ACAACCCCAAACTGACCAC-3′ and reverse 5′

72 -GGGCAGCAAATCAAAGGAG-3′

Sema3c: forward 5′-ACAAAGACAGGAGGAAGGAG-3′ and reverse 5′-AGTGGCAATGCAGTGGTAG-3′

Sema6a: forward 5′-GCTCACTCTATGTTGCATTCTC-3′ and reverse 5′-ACTTTCCCTTACCCACCCAC-3′

Il10: forward 5′-TGGGTGAGAAGCTGAAGACC-3′ and reverse 5′-TTCATGGCCTTGTAGACACC-3′

Ifng: forward 5′-ATCTGGAGGAACTGGCAAAA-3’ and reverse 5’-TTCAAGACTTCAAAGAGTCTGAGGTA -3′

Expression level of each gene was normalized to that of *Gapdh* and calculated relative to the expression in vehicle-treated group.

Measurement of IL-17 and PGE_2 concentration in culture supernatant of Th17 cells

IL-17 concentration in culture supernatant of differentiated Th17 cells stimulated with 100 μM db-cAMP, 10 μM FSK or 100 μM IBMX for 3 days was measured by a Mouse

IL-17 Quantikine ELISA Kit (M1700, R&D systems) according to the manufacturer's instruction.

Th17 cells were stimulated with 10 ng/ml IL-23 for 3 days in the absence or presence of 100 μM indomethacin and PGE_2 concentration in culture supernatant was determined by a Prostaglandin E_2 ELISA kit - monoclonal (514010, Cayman Chemical, Ann Arbor, MI) according to the manufacturer's instruction.

Gene expression of Th17 cells stimulated IL-23 and/or cAMP from microarray analysis

$CD4^+$ T cells were incubated in Th17-skewing condition for 4 days. Differentiated Th17 cells were then stimulated by IL-23, db-cAMP or combination for 24 h. RNA was purified with an RNeasy Mini Kit, amplified and revers transcribed by the High-capacity cDNA Reverse Transcription Kit. cDNA was fragmented and labeled by a Low Input Quick Amp Labeling Kit (Agilent, Santa Clara, CA), and then hybridized to a Gene Expression Large Volume Hybridization Kit (Agilent). Hybridized genes were

scanned by Gene chip scanner 3000 system. Data were analyzed by GeneSpring software (Agilent Technology, Santa Clara, CA).

Flow cytometry

The medium was removed after each incubation, and cells were re-stimulated with 50 ng/ml phorbol 12-myristate 13-acetate (PMA) (Sigma) and 500 ng/ml ionomycin (Sigma) in the presence of GolgiPlug (BD bioscience) for 4 h, followed by fixation and permeabilization with a fixation/permeabilization solution (Cytofix/Cytoperm, BD Pharmingen). Cells were then stained with anti-mouse CD45.2 antibody (eBioscience), anti-mouse CD4 antibody(BioLegend), anti-mouse IFN-γ antibody (eBioscience) and anti-mouse IL-17A antibody (BioLegend) followed by FACS analysis on LSR Fortessa (BD Bioscience).

Western blot analysis

Differentiated Th17 cells were cultured with 10 ng/ml IL-23 for 3 days to induce IL-23R, rested to return STAT3 phosphorylation to the basal level, and then re-stimulated with 100 μM db-cAMP and/or 100 ng/ml IL-23 for 30 min with indicated compounds for indicated time. Total cell lysates were prepared with RIPA buffer (Sigma) containing a phosphatase inhibitor cocktail (PhosSTOP, Roche, Basel, Switzerland) and a proteinase inhibitor cocktail (Complete Protease Inhibitor Cocktail, Roche). Lysates were then subjected to SDS-PAGE (sodium dodecyl sulfate-poly-acrylamide gel electrophoresis) and separated proteins were transferred to a PVDF membrane (Millipore, Darmstadt, Germany). After blocking with an ECL Blocking Agent (GE Healthcare, Piscataway, NJ), membranes were incubated with primary antibodies, followed by incubation with secondary antibodies conjugated with horseradish peroxidase (GE). Signals were detected using an ECL Prime Western Blotting Detection Reagent (GE) on LAS-4000 (GE). Primary antibodies used were; mouse monoclonal anti-α-Tubulin antibody (clone DM1A, #T6199, Sigma), mouse monoclonal anti-GAPDH antibody (clone 6C5, #AM4300, Ambion, Austin, TX), rabbit monoclonal anti-STAT3 antibody (clone 79D7, #4904, Cell Signaling Technology,

Danvers, MA), rabbit monoclonal anti-phosphorylated STAT3 antibody (Y705; #9145, Cell Signaling Technology), rabbit monoclonal anti-phosphorylated STAT3 antibody (S727; #9134, Cell Signaling Technology), rabbit monoclonal anti-JAK2 antibody (clone D2E12, #3230, Cell Signaling Technology), rabbit monoclonal anti-phosphorylated JAK2 antibody (Tyr1007/1008; #3771, Cell Signaling Technology), rabbit monoclonal anti-NF-κB p65 antibody (clone D14E12, #8242, Cell Signaling Technology), rabbit monoclonal anti-phosphorylated NF-κB p65 antibody (S536; clone 93H1, #3033, Cell Signaling Technology), rabbit monoclonal anti-p105/p50 antibody (#3035, Cell Signaling Technology), and rabbit monoclonal anti-phosphorylated p105 antibody (S933; #4086, Cell Signaling Technology).

RNA interference

siRNA for mouse *Creb1* (5′-UUGAACAACAACUUGGUUGCUGGGC-3′ (sense) or 5′-GCCCAGCAACCAAGUUGUUGUUCAA-3′ (antisense) and scrambled control siRNA were obtained from Invitrogen (Stealth RNAi, Carlsbad, CA). Th17 cells differentiated with TGF-β1 (1 ng/ml) and IL-6 (20 ng/ml) were transfected with 500

149 pmol of each siRNA using an Amaxa P3 Primary Cell 4D-Nucleofector X Kit with the
150 program DN100 on a 4D-Nucleofector (Lonza, Basel, Switzerland) in 100 μl. After
151 transfection for 4 h, cells were washed and stimulated with or without 10 ng/ml IL-23
152 for 2 days , and then incubated with or without 100 μM db-cAMP for 1 day. Total RNA
153 was prepared and then subjected to qRT-PCR analysis.

154

155 **Histology**

156 The ear tissues from psoriasis models were removed and fixed by 4 %
157 Paraformaldehyde (PFA) for 48h at 4°C. Each ear tissues were embedded in paraffin,
158 sectioned at 5 μm thickness, and then stained with hematoxylin-Eosin.

159

160 **Analysis of gene expression of human skin biopsies and mouse IL-23-treated ear**
161 **from microarray datasets**

162 Microarray gene expression data of human skin biopsies were retrieved from Gene
163 Expression Omnibus datasets (GSE51440 and GSE13355).[52,53] Patients information and

skin samples have been described previously.[52,53] In brief, two biopsies were taken from each patient - one from lesional skin of each patient (involved sample) and the other from non-lesional skin (uninvolved sample), taken at least 10 cm away from any active plaque. One biopsy was obtained from each healthy control.[52] Microarray gene expression data of IL-23-treated ear from mice was retrieved from GSE13335.[53] Gene expression levels were transformed to z-score values. *P* values were calculated by nonparametric Wilcoxon-Mann-Whitney test, and correlations between expression levels of two genes were calculated by nonparametric Spearman correlation test.

Statistical Analysis

Data are shown in mean ± SEM. Statistical comparisons among more than two groups were conducted using One-way ANOVA with Bonferoni test. Statistical comparisons between two groups were conducted using Mann-Whitney test. P values of 0.05 or less were considered significant.

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A_55_P2106043	Bsx
A_30_P01030933	
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ProbeName	GeneSymbol
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A_55_P2092310	Phgdh
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A_55_P1986306	Ltv1
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A_52_P518997	Epha2
A_52_P63343	Ciart
A_51_P510891	Afp
A_51_P514085	Mx2
A_52_P547662	P2ry1
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A_51_P254234	Chchd4
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A_55_P2026818	Slc4a7
A_55_P2046262	Phgdh
A_51_P502152	Slc19a1
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A_55_P2085142	Spp1
A_55_P1960916	Egln3
A_51_P156434	Slc25a33
A_55_P2074736	Prkar1b
A_65_P19933	Zdhhc23
A_55_P1964302	Timm8a1
A_55_P2110037	Akap7
A_51_P317443	Cd3eap
A_51_P518163	Rrp9
A_51_P242859	Akr1c12

A_55_P2002968	Coro2a
A_52_P406828	Dkc1
A_51_P291749	Pecr
A_52_P131548	Ajuba
A_55_P2066463	Enah
A_55_P1987186	Ttll9
A_55_P2021149	Cltb
A_66_P127160	Eif2b3
A_55_P2090254	Sntg2
A_55_P1953819	Btk
A_52_P20906	Twist1
A_51_P187901	Nop56
A_52_P416327	Cd226
A_52_P246703	Ak7
A_30_P01021097	
A_55_P1960023	Trmt5
A_55_P2006118	Rbp4
A_51_P183894	Fbxo15
A_55_P2019312	Car12
A_55_P1988384	Slc7a3
A_65_P08971	F3
A_51_P172251	Ifrd2
A_55_P2044917	Gpr83
A_55_P1972575	Tmeff1
A_52_P651948	Fam229b
A_51_P390538	Mpeg1
A_66_P116678	Rps8
A_51_P230507	Ell2
A_51_P300709	Srm
A_55_P1973941	Slc7a5
A_55_P2062627	Tmem238
A_51_P170758	Grwd1
A_51_P358722	Lancl3
A_55_P2158873	Ppid
A_51_P401527	Rnmtl1
A_55_P2000182	Slc5a6
A_52_P196979	Trim66
A_55_P1981994	Krt17
A_55_P1966573	Gemin4
A_51_P186053	Rrp15

A_55_P2021505	Adra2a
A_66_P129111	Nasp
A_51_P466162	C1qbp
A_51_P468249	Phex
A_55_P1996354	Wdr31
A_51_P431737	Cth
A_55_P2143311	Pdxk
A_51_P238383	B4galt4
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A_55_P2235931	Cacna1c
A_55_P2011220	Armcx1
A_55_P2128929	Cc2d2a
A_55_P2061084	Wfikkn2
A_66_P136228	Rai14
A_55_P2033725	Ascl2
A_52_P359965	Cpd
A_51_P114462	Ccl17
A_66_P109192	Gm2464
A_52_P400677	AW209491
A_51_P485458	Txlna
A_55_P2057035	Slmo1
A_55_P2029319	Cd70
A_55_P1969481	Hivep3
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A_55_P1971889	F3
A_55_P2030486	Srsf2
A_55_P2002376	Srm
A_52_P127682	Dagla
A_51_P261164	F2rl2
A_51_P410451	Tube1
A_51_P117865	Fam20c
A_51_P513992	Spag4
A_52_P452689	Atf3
A_52_P93910	Nrp2
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A_51_P346641	Armcx4
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A_52_P344290	F2r
A_52_P596755	Dnph1
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A_51_P164420	Eef1e1
A_55_P2063096	Txnrd3
A_55_P1978441	Kif3a
A_51_P205779	Cd5l
A_51_P431734	Fam185a
A_52_P543040	Utp14a
A_55_P2143041	Phgdh
A_55_P1992834	Socs2
A_52_P673499	Shmt1
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A_51_P335758	Chn1
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A_52_P58949	Calcrl
A_52_P97699	D430019H16Rik
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A_55_P2016249	Ppid
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A_51_P134812	Chac1
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A_51_P164203	Nme4
A_51_P296487	Lss
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A_51_P125567	Mettl13
A_52_P481279	Drc1
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A_55_P2193512	Cd226
A_55_P1952744	Timm8a1
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A_55_P2115225	Fap
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A_52_P152631	Tmem17
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A_30_P01030879	
A_51_P156438	Slc25a33
A_55_P1978052	Pet112
A_52_P248403	
A_55_P2033680	
A_55_P2168823	
A_55_P2083449	Spryd7
A_55_P2042486	Dpysl3
A_55_P2080168	Dgkk
A_51_P205326	Fam198a
A_55_P1989102	Hmgn1
A_55_P2137527	Fam183b
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A_55_P2184370	LOC102641088

A_51_P455647	Car2
A_55_P1964093	Rangrf
A_55_P1973995	Gm6756
A_55_P2128085	LOC102641654
A_52_P853177	Angptl2
A_52_P625640	Trim9
A_55_P1958857	Nek6
A_52_P231075	Fcrls
A_51_P401907	Gm5483
A_55_P1992490	Scg2
A_55_P1958464	
A_51_P150302	Crtam
A_55_P2134800	Cinp

ProbeName	GeneSymbol
A_51_P385099	Tnf
A_55_P1967514	Dnah7a
A_55_P2103698	Isg15
A_55_P2122075	Pdcd1lg2
A_55_P2165234	2300005B03Rik
A_55_P2187141	Pdcd1lg2
A_51_P160344	Cenpv
A_55_P2012989	Slamf7
A_55_P2163138	Tm4sf5
A_52_P157880	
A_55_P2138386	Il5
A_51_P242166	Lap3
A_55_P2205858	Col6a5
A_55_P2079020	Snhg7os
A_66_P101942	Gm9706
A_55_P2179463	Tnfsf8
A_51_P496432	Acsl1
A_51_P188281	Myf5
A_51_P113178	Fam212b
A_55_P2125972	Gorasp1
A_51_P272563	Naa25
A_66_P117543	
A_55_P1964559	Smarca5-ps
A_51_P187018	Magohb
A_52_P649561	Heg1
A_51_P270426	Egr4
A_55_P2085974	Igf1
A_52_P344978	
A_51_P184728	Cnksr3
A_66_P105460	Ccdc14
A_51_P273609	Itpka
A_30_P01028030	
A_66_P128631	Cinp
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A_51_P308844	Nrn1
A_52_P13897	Hook1
A_52_P639774	Gart
A_66_P128537	Isg15
A_51_P228768	Slfn3
A_51_P516085	Dntt
A_55_P2134804	Cinp
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A_55_P2084703	Acaca
A_51_P234627	Nubpl
A_55_P2172182	Olfr1138
A_55_P2004452	Tceal8
A_55_P2128144	Il19
A_51_P200561	4930506M07Rik
A_51_P165098	Gga2
A_52_P987201	Pdzrn4
A_55_P1964960	Il33
A_55_P2123037	Olfr553
A_51_P301215	Knop1
A_30_P01025391	
A_51_P232399	Acy3
A_55_P2039684	Gpr34
A_51_P347452	Htatsf1
A_51_P215530	Rnf180
A_52_P131836	Bysl
A_55_P2082806	Trib1
A_55_P2095880	Nfix

GO ACCESSION	GO Term	p-value	corrected p-value	-logP	gene
GO:0031347	regulation of defense response	2.87E-08	1.64E-04	3.78E+00	Il1r1 Il17f Foxf1 Calca Il17a Il23r
GO:0032101	regulation of response to external stimulus	1.03E-07	1.97E-04	3.71E+00	Il1r1 Il17f Foxf1 Calca Il17a Il23r
GO:0050727	regulation of inflammatory response	7.57E-08	1.97E-04	3.71E+00	Il1r1 Il17f Foxf1 Calca Il17a
GO:0080134	regulation of response to stress	1.28E-06	0.00183381	2.74E+00	Il1r1 Il17f Foxf1 Calca Il17a Il23r

GO:1900017	positive regulation of cytokine production involved in inflammatory response	7.20E-06	0.008231741	2.08E+00	Il17f Il17a
GO:1900015	regulation of cytokine production involved in inflammatory response	1.54E-05	0.014684372	1.83E+00	Il17f Il17a
GO:0006954	inflammatory response	2.83E-05	0.020216491	1.69E+00	Il17f Calca Il17a Il23r
GO:0071345	cellular response to cytokine stimulus	2.55E-05	0.020216491	1.69E+00	Il1r1 Foxf1 Il17a Il23r

GO:0031328	positive regulation of cellular biosynthetic process	6.72E-05	0.029578676	1.53E+00	Nr2e3 Il17f Mycn Foxf1 Calca Il17a
GO:0034097	response to cytokine	5.94E-05	0.029578676	1.53E+00	Il1r1 Foxf1 Il17a Il23r
GO:0045935	positive regulation of nucleobase-containing compound metabolic process	4.74E-05	0.029578676	1.53E+00	Nr2e3 Il17f Mycn Foxf1 Calca Il17a
GO:0045944|GO:0010552|GO:0045817	positive regulation of transcription from RNA polymerase II promoter	6.37E-05	0.029578676	1.53E+00	Nr2e3 Il17f Mycn Foxf1 Il17a

GO:0051173	positive regulation of nitrogen compound metabolic process	6.47E-05	0.029578676	1.53E+00	Nr2e3 Il17f Mycn Foxf1 Calca Il17a
GO:0009891	positive regulation of biosynthetic process	7.62E-05	0.03114245	1.51E+00	Nr2e3 Il17f Mycn Foxf1 Calca Il17a
GO:0033993	response to lipid	8.48E-05	0.032340873	1.49E+00	Nr2e3 Rbp1 Il17a Il23r

GO ACCESSION	GO Term	p-value	corrected p-value	-logP	gene
GO:0005615	extracellular space	8.63E-06	0.0250513	1.6011702	Lum Tgfbi Wnt6 Timp1 Crispld2
GO:0031012	extracellular matrix	8.10E-06	0.0250513	1.6011702	Lum Tgfbi Wnt6 Il1rn Timp1 Enpp2 Il22

GO ACCESSION	GO Term	p-value	corrected p-value	-logP	
GO:0044459	plasma membrane part	3.77E-10	1.73E-05	4.76E+00	Cacna1s Synpo Tgfb3 Ms4a4b Cd59a Hc Il7r Selp Cd27 Ccr2 Lag3 Cd160 Sytl2 Gpnmb Gabbr1 Vmn1r24 Gpc3 Sema4f Cd24a Klrc1 Pde4b Ms4a1 Ntrk3 S1pr1 Itgb3 Slc22a22 Nt5e Cxcr4 Pla2g4f Ifnlr1 Camk2n1 Abca1 Plaur Atp6v0d2 Dcc Tdgf1 Shc4 Rasa3 Catsper3 Atp2b2 Sgcg Il12rb2 Abcb4 Grm1 Slc8a3 Shank2 Cacna1h Gria4 Ms4a4b Scn3b Klra4 Klra1 Klrc1 Ntrk3 Cx3cr1 Slc17a3 Sell Pde4d Fasl Lzts3 Slco6b1 Trpm6 Kcng1 Ntrk3 Sgip1 Vmn1r203 Tmprss11e Kcnmb4 Adra1b P2rx4 Adora2a Sipa1l1 Gabra3 Klrd1 Klri2 Smo Slc52a3 Sdcbp Wwc1 Cyth3 Klrc3 Dmd Akap7 Clstn3 Cacnb2 Catsperd Kcnma1 Igf1r Cd33 Kctd12 Atp6v0d2 Tlr4

GO:0071944	cell periphery	1.27E-08	2.92E-04	3.53E+00	Cacna1s Gpr65 Arrdc4 Synpo Tgfb3 Cysltr2 Ms4a4b Cd59a Hc Amigo2 Slc46a2 Il7r Abcb9 Ptprz1 Selp Cd27 Lat2 Ccr2 Lag3 Cd160 Sytl2 Tmod1 Cpm Olfr1414 Grb7 Tspan2 Rab19 Olfr983 Paqr8 Gpnmb Khdc3 Cldn10 Gabbr1 Olfr959 Il1r2 Vmn1r24 Gpr171 Prnp Sptb Slc16a9 Bst1 Gabbr1 Ramp1 Vcl Tbc1d30 Fzd10 Gpc3 Sema4f Cd24a Magi1 Klrc1 Svil Pde4b Fam110c Ms4a1 Ntrk3 Klra15 S1pr1 Itgb3 Sla2 Slc22a22 Nt5e Cxcr4 Pla2g4f Ifnlr1 Camk2n1 Abca1 Plaur Atp6v0d2 Txk Ramp3 Igflr1 Lsp1 Klra16 P2ry14 Pigr Dcc Cxcr6 Tdgf1 Shc4 Rasa3 Catsper3 Atp2b2 Olfr668 Sgcg Il12rb2 Abcb4 Grm1 Slc8a3 Shank2 Cacna1h Gria4 Ms4a4b Vmn2r48 Rasa3 Ptprz1 Scn3b Cd300lf Klra4 Klra1 Klrc1 Olfr1446 Ntrk3 Dgkb Cx3cr1 Vmn2r91 Slc17a3 Sell Pde4d Atp2b3 Olfr54 Wwox Fasl Cap2 Samhd1 Lzts3 Slc16a5 Slco6b1 Gpr146 Tspan2 Cdcp1 Trpm6 Piezo2 Gpr82 Klra23 Efna2 Cx3cr1 Kcng1 Arl4d Fgfr1 Sgip1 Pde6a Inadl Vipr1 Vmn1r203 Ptpn13 Vmn2r60 Tmprss11e Olfr1320 Nid1 Ifitm1 Kcnmb4 Olfr701 Adra1b Ramp3 P2rx4 Vmn2r96 Pde4b Art4 Adora2a Sipa1l1 Gabra3 Klrd1 Klri2 Smo Prrt1 Slc52a3 Slc16a2 Sdcbp Wwc1 Ermn Rasgrp2 Ifitm1 Cyth3 Zan Cat Klrc3 Sema6c Gpr114 Thsd7a Ntn1 Cxcr6 Dmd Cacna1s Ptpn13 Akap7 Olfr566 Clstn3 Mrgprg Cfh Cacnb2 Dgkg Snap23 Caln1 Catsperd Kcnma1 Pde4b Pde4d Olfr726 Igf1r Cd33 Cdh18 Kctd12 Atp6v0d2 Tlr4 Itgb3

GO:0005886|GO:0005904	plasma membrane	7.45E-08	0.0011393	2.94E+00	Cacna1s Gpr65 Arrdc4 Synpo Tgfb3 Cysltr2 Ms4a4b Cd59a Hc Amigo2 Slc46a2 Il7r Abcb9 Ptprz1 Selp Cd27 Lat2 Ccr2 Lag3 Cd160 Sytl2 Cpm Olfr1414 Grb7 Tspan2 Rab19 Olfr983 Paqr8 Gpnmb Cldn10 Gabbr1 Olfr959 Il1r2 Vmn1r24 Gpr171 Prnp Sptb Slc16a9 Bst1 Gabbr1 Ramp1 Vcl Tbc1d30 Fzd10 Gpc3 Sema4f Cd24a Magi1 Klrc1 Svil Pde4b Ms4a1 Ntrk3 Klra15 S1pr1 Itgb3 Sla2 Slc22a22 Nt5e Cxcr4 Pla2g4f Ifnlr1 Camk2n1 Abca1 Plaur Atp6v0d2 Txk Ramp3 Igflr1 Lsp1 Klra16 P2ry14 Pigr Dcc Cxcr6 Tdgf1 Shc4 Rasa3 Catsper3 Atp2b2 Olfr668 Sgcg Il12rb2 Abcb4 Grm1 Slc8a3 Shank2 Cacna1h Gria4 Ms4a4b Vmn2r48 Rasa3 Ptprz1 Scn3b Cd300lf Klra4 Klra1 Klrc1 Olfr1446 Ntrk3 Dgkb Cx3cr1 Vmn2r91 Slc17a3 Sell Pde4d Atp2b3 Olfr54 Wwox Fasl Cap2 Samhd1 Lzts3 Slc16a5 Slco6b1 Gpr146 Tspan2 Cdcp1 Trpm6 Piezo2 Gpr82 Klra23 Efna2 Cx3cr1 Kcng1 Ntrk3 Arl4d Fgfr1 Sgip1 Pde6a Inadl Vipr1 Vmn1r203 Ptpn13 Vmn2r60 Tmprss11e Olfr1320 Ifitm1 Kcnmb4 Olfr701 Adra1b Ramp3 P2rx4 Vmn2r96 Pde4b Art4 Adora2a Sipa1l1 Gabra3 Klrd1 Klri2 Smo Prrt1 Slc52a3 Slc16a2 Sdcbp Wwc1 Rasgrp2 Ifitm1 Cyth3 Zan Cat Klrc3 Sema6c Gpr114 Thsd7a Cxcr6 Dmd Cacna1s Ptpn13 Akap7 Olfr566 Clstn3 Mrgprg Cfh Cacnb2 Dgkg Snap23 Caln1 Catsperd Kcnma1 Pde4b Pde4d Olfr726 Igf1r Cd33 Cdh18 Kctd12 Atp6v0d2 Tlr4 Itgb3

GO:0009897	external side of plasma membrane	2.16E-07	0.0024761	2.61E+00	Cd59a Il7r Selp Cd27 Ccr2 Lag3 Cd24a Klrc1 Ms4a1 S1pr1 Itgb3 Cxcr4 Abca1 Il12rb2 Klra4 Klra1 Klrc1 Sell Fasl Klrd1 Klrc3 Kcnma1 Cd33 Tlr4 Itgb3
GO:1902531|GO:0010627	regulation of intracellular signal transduction	1.46E-06	0.0134296	1.87E+00	Dusp10 Rapgef3 Tgfb3 Cysltr2 Phlpp1 Tnip3 Selp Cd27 Sesn3 Xdh Tgfb2 Il20ra Ecm1 Sfrp4 Prnp Hgf Il18r1 Bcl6 Fzd10 Cd24a Gcnt2 Fam110c Ntrk3 Itgb3 Sla2 Hes5 Abca1 Dcc Tdgf1 Agpat9 Rasa3 Grm1 Rasa3 Dusp7 Ntrk3 Pde4d Tnfaip3 Ntrk3 Fgfr1 C1qtnf3 Dusp5 Rora Il18r1 Adra1b P2rx4 Sipa1l1 Klf4 Sipa1l2 Wwc1 Sipa1l2 Rasgrp2 Cyth3 Cat Cmya5 Dmd Akap7 Tgfb2 Pde4d Ecm1 Igf1r Tlr4 Itgb3

GO:0002376	immune system process	3.03E-06	0.0213073	1.67E+00	Klf2 Hc Slc46a2 Il7r Tnip3 Ptprz1 Selp Cd27 Lat2 Ccr2 Epas1 Tgfb2 Procr Tspan2 Foxj1 Sptb Il18r1 Bcl6 Gcnt1 Gpc3 Cxcl3 Cd24a Tcf7 Pde4b Ms4a1 S1pr1 Sla2 Tlx1 Cxcr4 Ifnlr1 Txk P2ry14 Pigr Tdgf1 Slc8a3 Ctse Ptprz1 Cd300lf Cx3cr1 Pde4d Fasl Myb Samhd1 Tspan2 Gab3 Efna2 Tnfaip3 Cx3cr1 Rora Pglyrp1 Gcnt1 Ifitm1 Il18r1 Foxp1 Pde4b Tcf7 Eml1 Spib Klf4 Serpinb9 Eml1 Ifitm1 Aicda Eomes Cfh Ifnar1 Ifnar1 Pde4b Tgfb2 Pde4d Cblb Runx2 Igf1r Tlr4
GO:0004896|GO:0004907	cytokine receptor activity	3.25E-06	0.0213073	1.67E+00	Il7r Ccr2 Il1r2 Il18r1 Il18rap Cxcr4 Ifnlr1 Cxcr6 Il12rb2 Cx3cr1 Cxcr6 Ifnar1

GO:0009653	anatomical structure morphogenesis	5.97E-06	0.0339258	1.47E+00	Cacna1s Rapgef3 Tgfb3 Klf2 Serpinb5 Il7r Ptprz1 Selp Ptprb Ccr2 Epas1 Nyap2 mod1 Tgfb2 Ecm1 Foxj1 Sfrp4 Spaca1 Matn2 Hgf Col4a2 Bcl6 Ramp1 Vcl Gcnt1 Map2 Gpc3 Sema4f Tcf7 Ablim1 Nr4a2 S1pr1 Itgb3 Tlx1 Cxcr4 Murc Dcc Tdgf1 Atp2b2 Spaca1 Sall3 Gcm1 Ptprz1 Nr2e1 Cryaa Tead1 Wwox Fasl Cap2 Efna2 Fgfr1 Tbx6 Dusp5 Rora Gcnt1 Ifitm1 Hmx2 Foxp1 Adora2a Tcf7 Ctnnd2 Tmem106b Smo Klf4 Slitrk4 Ermn Flrt3 Ifitm1 Gcm1 Sema6c Thsd7a Ntn1 Dmd Eomes Runx2 Igf1r Crispld1
GO:0005891	voltage-gated calcium channel complex	6.66E-06	0.0339258	1.47E+00	Cacna1s Pde4b Catsper3 Cacna1h Pde4d Cacnb2 Catsperd

GO:0051239	regulation of multicellular organismal process	8.09E-06	0.0371204	1.43E+00	Dusp10 Rapgef3 Tgfb3 Cysltr2 Cd59a Klf2 Hc Slc46a2 Il7r Ptprz1 Selp Lama4 Cd27 Ccr2 Epas1 Aspa Lag3 Sytl2 Proc Xdh Tgfb2 Il20ra Procr Ecm1 Foxj1 Sfrp4 Prnp Hgf Col4a2 Il18r1 Tg Bcl6 Gpc3 Sema4f Cd24a Gcnt2 Pde4b Nr4a2 Ntrk3 S1pr1 Itgb3 Cxcr4 Hes5 Txk Dcc Tdgf1 Il12rb2 Grm1 Ptprz1 Scn3b Nr2e1 Mfap4 Ntrk3 Cx3cr1 Pde4d Fasl Myb Tnfaip3 Fgfr1 Sgip1 Tbx6 C1qtnf3 Rora Pglyrp1 Tnnt3 Adra1b P2rx4 Foxp1 Pde4b Adora2a Sipa1l1 Smo Klf4 Cmya5 Ntn1 Dmd Btg1 Eomes Kcnma1 Ifnar1 Cblb Runx2 Ccnd1 Tlr4
GO:0007166	cell surface receptor signaling pathway	8.93E-06	0.0372139	1.43E+00	Tgfb3 Il7r Wisp1 Cd27 Lat2 Ccr2 Lag3 Clnk Tgfb2 Il20ra Sfrp4 Il1r2 Hgf Il18r1 Fzd10 Cd24a Tcf7 Gcnt2 Pde4b Ntrk3 Il18rap Itgb3 Cxcr4 Ifnlr1 Hes5 Abca1 Plaur Txk P2ry14 Pigr Dcc Cxcr6 Tdgf1 Adamts14 Il12rb2 Grm1 Gria4 Adam34 Ntrk3 Cx3cr1 Wwox Fasl Kremen1 Efna2 Cx3cr1 Ntrk3 Fgfr1 Vipr1 Tle2 P2rx4 Pde4b Adora2a Tcf7 Sipa1l1 Smo Wisp1 Eya2 Gpr114 Cxcr6 Akap7 Ifnar1 Cblb Runx2 Igf1r Ccnd1 Tlr4

GO:0048583	regulation of response to stimulus	1.02E-05	0.0389147	1.41E+00	Dusp10 Rapgef3 Tgfb3 Cysltr2 Cd59a Hc Phlpp1 Il7r Tnip3 Abcb9 Selp Cd27 Lat2 Ccr2 Sesn3 Lag3 Proc Xdh Tgfb2 Il20ra Grb7 Foxj1 Sfrp4 Prnp Hgf Il18r1 Bcl6 Ramp1 Fzd10 Gpc3 Cd24a Gcnt2 Pde4b Fam110c Ntrk3 S1pr1 Itgb3 Sla2 Nt5e Cxcr4 Ifnlr1 Hes5 Abca1 Txk Ramp3 Pigr Dcc Tdgf1 Agpat9 Rasa3 Bicc1 Sall3 Grm1 Dusp7 Cx3cr1 Sell Pde4d Wwox Fasl Myb Samhd1 Tnfaip3 Fgfr1 Zfyve28 C1qtnf3 Dusp5 Padi2 Rora Pglyrp1 Tle2 Adra1b Ramp3 P2rx4 Adora2a Sipa1l1 Klf4 Sipa1l2 Wwc1 Rasgrp2 Cyth3 Cat Cmya5 Dmd Akap7 Cfh Pde4d Cblb Runx2 Ecm1 Igf1r Ccnd1 Kctd12 Tlr4
GO:0098552	side of membrane	1.38E-05	0.0486405	1.31E+00	Cd59a Il7r Selp Cd27 Ccr2 Lag3 Cd24a Klrc1 Ms4a1 S1pr1 Itgb3 Cxcr4 Abca1 Rasa3 Il12rb2 Klra4 Klra1 Klrc1 Sell Fasl Klrd1 Cyth3 Klrc3 Kcnma1 Cd33 Tlr4

SUPPLEMENTARY FIGURES LEGENDS

Figure E1. **Effects of COX inhibitors on *Il23r* expression.** (A) Expression of *Il23r* in differentiated Th17 cells treated with IL-23 (10 ng/ml) and/or PGE_2 (100 nM) in the absence or presence of a COX-1 inhibitor, SC-560 (100 μM) or a COX-2 inhibitor, SC-236 (100 μM) or both for 3 days. (B) Th17 cells were cultured with vehicle, IL-23 (10 ng/ml) and PGE_2 (100 nM) or IL-23 (10 ng/ml), EP2 agonist (100 nM) and EP4 agonist (100 nM) in the absence or presence of indomethacin (100 μM) for 3 days, and then harvested to analyze for *Il23r* expression by qRT-PCR.

Figure E2. **db-cAMP activates JAK2 and STAT3 in Th17 cells.** (A) Time-course of STAT3 Y705 phosphorylation by vehicle, db-cAMP, IL-23 or db-cAMP and IL-23 in Th17 cells. Th17 cells were treated with IL-23 (10 ng/ml) for 3 days to induce IL-23R expression. The cells were then stimulated with either 100 μM db-cAMP or 100 ng/ml IL-23 or in combination for indicated times. Phosphorylation of STAT3 at Y705 residues and S727 residues under each condition was examined at indicated times by Western blot analysis using total cell lysates and antibodies to each phosphorylation site

of STAT3, total STAT3 and α-Tubulin. Representative images are shown (n=2). (B) Involvement of Src family kinase in cAMP-induced JAK2 Y1007/Y1008 phosphorylation. Th17 cells were stimulated with 100 μM db-cAMP for 10 min in the presence of a Src inhibitor, Src Kinase Inhibitor I (10 μM), and subjected to Western blot analysis. Data was from a single experiment.

Figure E3. **Heat-map and gene ontology analysis of genes in each cluster.** (A) Gene expression profiles in Th17 cells stimulated with db-cAMP and/or IL-23 followed by microarray analysis. Heat-map analysis of expression of genes 2-folds up- or down-regulated upon each stimulus compared to the vehicle control (One-way ANOVA $p<0.05$, n=3). (B) Gene ontology analysis of each clusters by GeneSpring.

Figure E4. **Involvement of PGE_2 signaling in psoriasis-like model.** (A) Gene expression of PGE_2 synthases in ear skin from naïve WT mice or psoriasis-like skin lesions from mice administrated with IL-23 by intradermal injection in the dorsum (n=5 each). Gene expression was retrieved from a public dataset GSE13335. (B and C)

Genetic loss of *Ptger2* (EP2 KO) or pharmacological EP4 antagonism alone does not cause alteration in the ear. Psoriasis-like model in WT and EP2 KO mice were established as described in Figure 5A. Ear swelling was measured every 2 days (B) (n=14, 10, 8, and 10 in vehicle-treated mice, EP2 KO mice, AS1954813-treated WT mice, AS1954813-treated EP2 KO mice, respectively) and ear skins were subjected to FACS analysis on day 4 (C). (D and E) Suppression of IL-17A$^+$ and IL-17A$^+$IFN-γ^+ CD4$^+$ T cell accumulation by EP2 KO and EP4 antagonist. WT and EP2 KO mice were administered either vehicle or AS1954813, and subcutaneously injected IL-23. CD4$^+$ T cells were purified from the ear of each group on day 4 and examined by FACS for IL-17A and IFN-γ. Representative data from 4 independent experiments are shown. All bars indicate mean ± SEM. *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$.

Figure E5. **FACS analysis of cell populations of EP2$^{fl/fl}$EP4$^{fl/fl}$Lck-Cre$^+$ mice or WT Lck-Cre$^+$ mice.** (A) Cell population of EP2$^{fl/fl}$EP4$^{fl/fl}$Lck-Cre$^+$ mice and control WT Lck-Cre$^+$ mice. The numbers of B cell, T cell, CD4 T cell, CD8 T cell, Th1 cell, Th17 cell and Treg cell isolated from thymus, spleen, lymph node, and peripheral blood were

analyzed by FACS. (n=3-4)

Figure E6. **Involvement of PGE_2 signaling in IMQ-induced psoriasis-like model and the effect of COX inhibitors on IL-23-induced psoriasis model.** (A) Control WT mice and *Ptger2*-deficient (EP2 KO) mice were subjected to imiquimod (IMQ)-induced psoriasis model and administered either vehicle or AS1954813, 100 mg/kg, as described in Figure 5A. Ear swelling was measured every 2 days (A) (n=14, 10, 8, and 10 in vehicle-treated mice, EP2 KO mice, AS1954813-treated WT mice, AS1954813-treated EP2 KO mice, respectively). (B-D) Female WT mice were subjected to IL23-induced psoriasis model and administered either vehicle, SC-236 (10mg/kg) or indomethacin (4 mg/kg). Ear swelling was measured every 2 days (B) (n=4, respectively) and mice were sacrificed and subjected to FACS analysis at day 4 (C and D).

Figure E7. **IL-1β-IL1 receptor signaling was not involved in *Il23r* expression by Th17 cells.** Expression of *Il23r* gene in differentiated Th17 cells stimulated with db-cAMP, IL-23 or db-cAMP and IL-23 in combination in the absence or presence of

65 various concentrations of neutralization antibody for IL-1β for 3 days was analyzed by

66 qRT-PCR (n=3). All bars indicate mean ± SEM.

67

68 Table E 1. **List of genes in Cluster 1U.**

69

70 Table E 2. **List of genes in Cluster 2U.**

71

72 Table E 3. **List of genes in Cluster 3U.**

73

74 Table E 4. **List of genes in Cluster 4U.**

75

76 Table E 5. **List of genes in Cluster 1D.**

77

78 Table E 6. **List of genes in Cluster 2D.**

79

80 Table E 7. **List of genes in Cluster 3D.**

Table E 8. **List of genes in Cluster 4D.**

Table E 9. **List of gene ontology from Cluster 1U.**

Table E 10. **List of gene ontology from Cluster 2U.**

Table E 11. **List of gene ontology from Cluster 3U.**

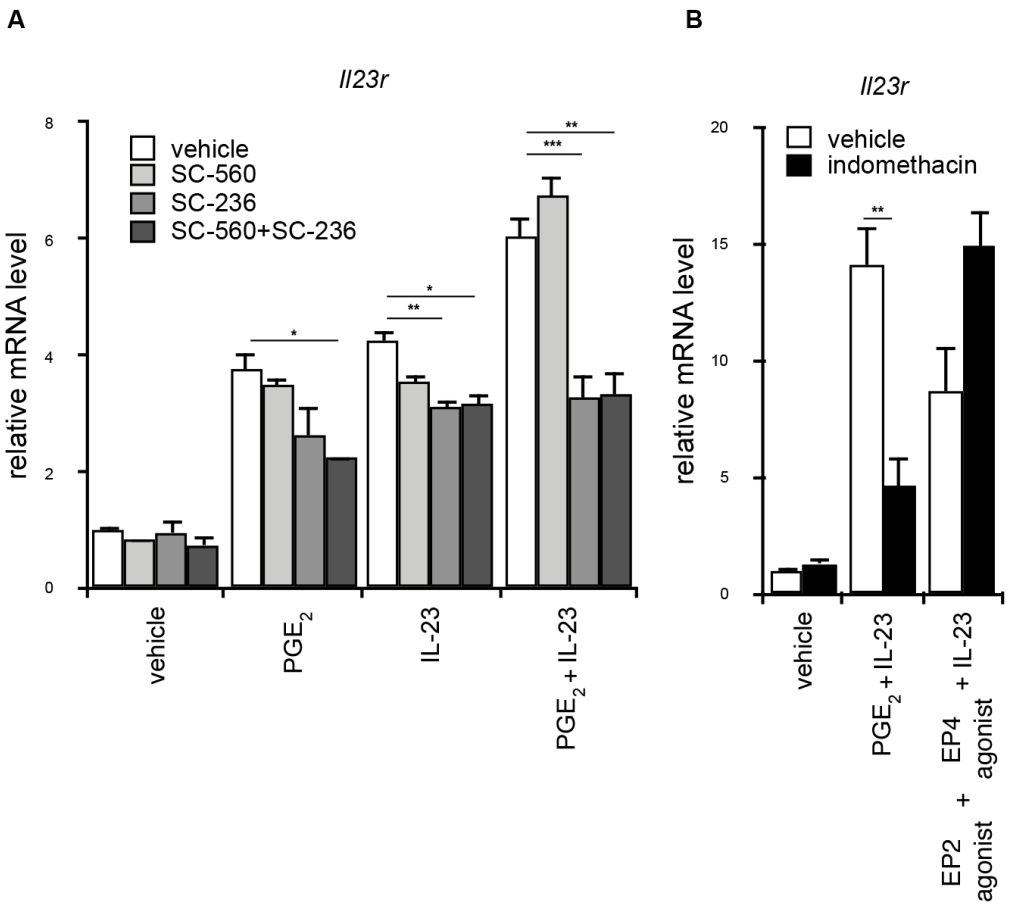
A
Il23r
relative mRNA level
vehicle
SC-560
SC-236
SC-560+SC-236
vehicle
PGE2
IL-23
PGE2 + IL-23
B
Il23r
relative mRNA level
vehicle
indomethacin
vehicle
PGE2 + IL-23
EP2 agonist + EP4 agonist + IL-23

Figure E1

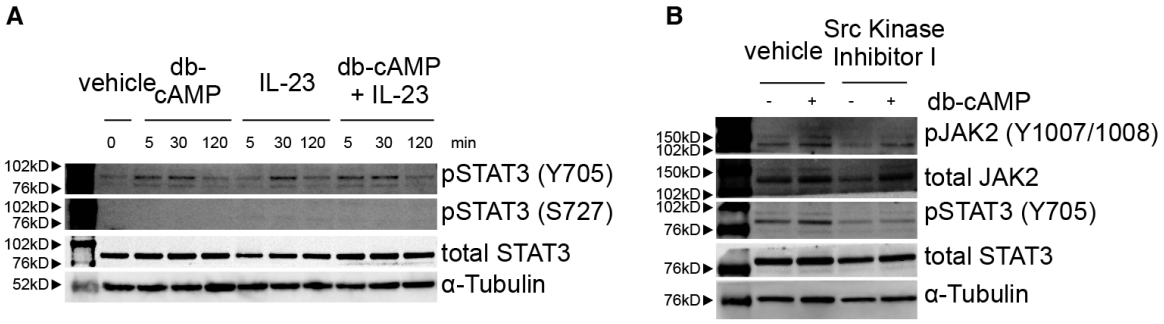
A
vehicle
db-cAMP
IL-23
db-cAMP + IL-23
0 5 30 120 5 30 120 5 30 120 min
102kD
76kD
pSTAT3 (Y705)
pSTAT3 (S727)
total STAT3
52kD
α-Tubulin
B
vehicle
Src Kinase Inhibitor I
- + - +
db-cAMP
150kD
102kD
pJAK2 (Y1007/1008)
total JAK2
pSTAT3 (Y705)
total STAT3
α-Tubulin

Figure E2

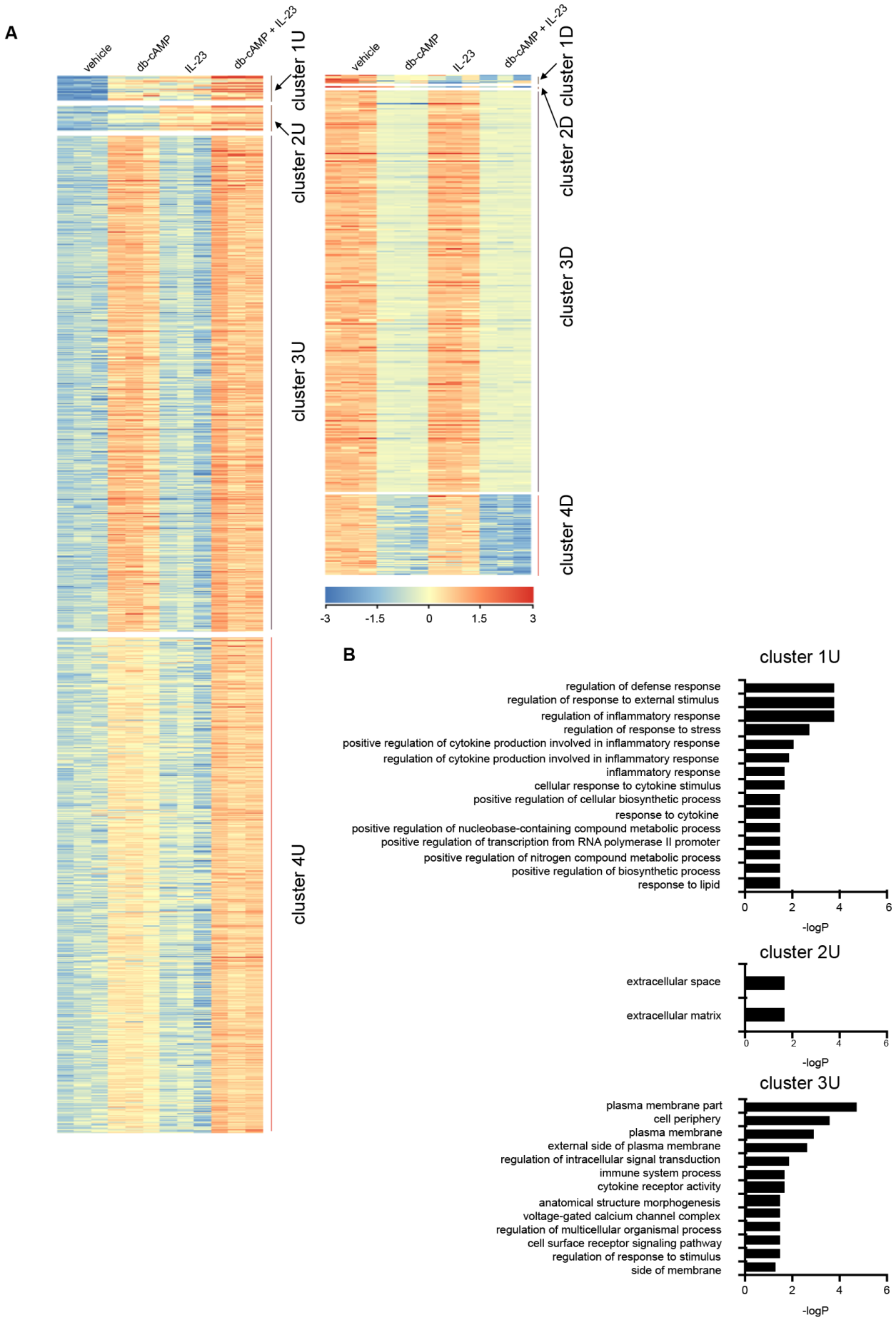
A
vehicle
db-cAMP
IL-23
db-cAMP + IL-23
cluster 1U
cluster 2U
cluster 3U
cluster 4U
cluster 1D
cluster 2D
cluster 3D
cluster 4D
-3
-1.5
0
1.5
3
B
cluster 1U
regulation of defense response
regulation of response to external stimulus
regulation of inflammatory response
regulation of response to stress
positive regulation of cytokine production involved in inflammatory response
regulation of cytokine production involved in inflammatory response
inflammatory response
cellular response to cytokine stimulus
positive regulation of cellular biosynthetic process
response to cytokine
positive regulation of nucleobase-containing compound metabolic process
positive regulation of transcription from RNA polymerase II promoter
positive regulation of nitrogen compound metabolic process
positive regulation of biosynthetic process
response to lipid
0
2
4
6
-logP
cluster 2U
extracellular space
extracellular matrix
0
2
4
6
-logP
cluster 3U
plasma membrane part
cell periphery
plasma membrane
external side of plasma membrane
regulation of intracellular signal transduction
immune system process
cytokine receptor activity
anatomical structure morphogenesis
voltage-gated calcium channel complex
regulation of multicellular organismal process
cell surface receptor signaling pathway
regulation of response to stimulus
side of membrane
0
2
4
6
-logP

Figure E3

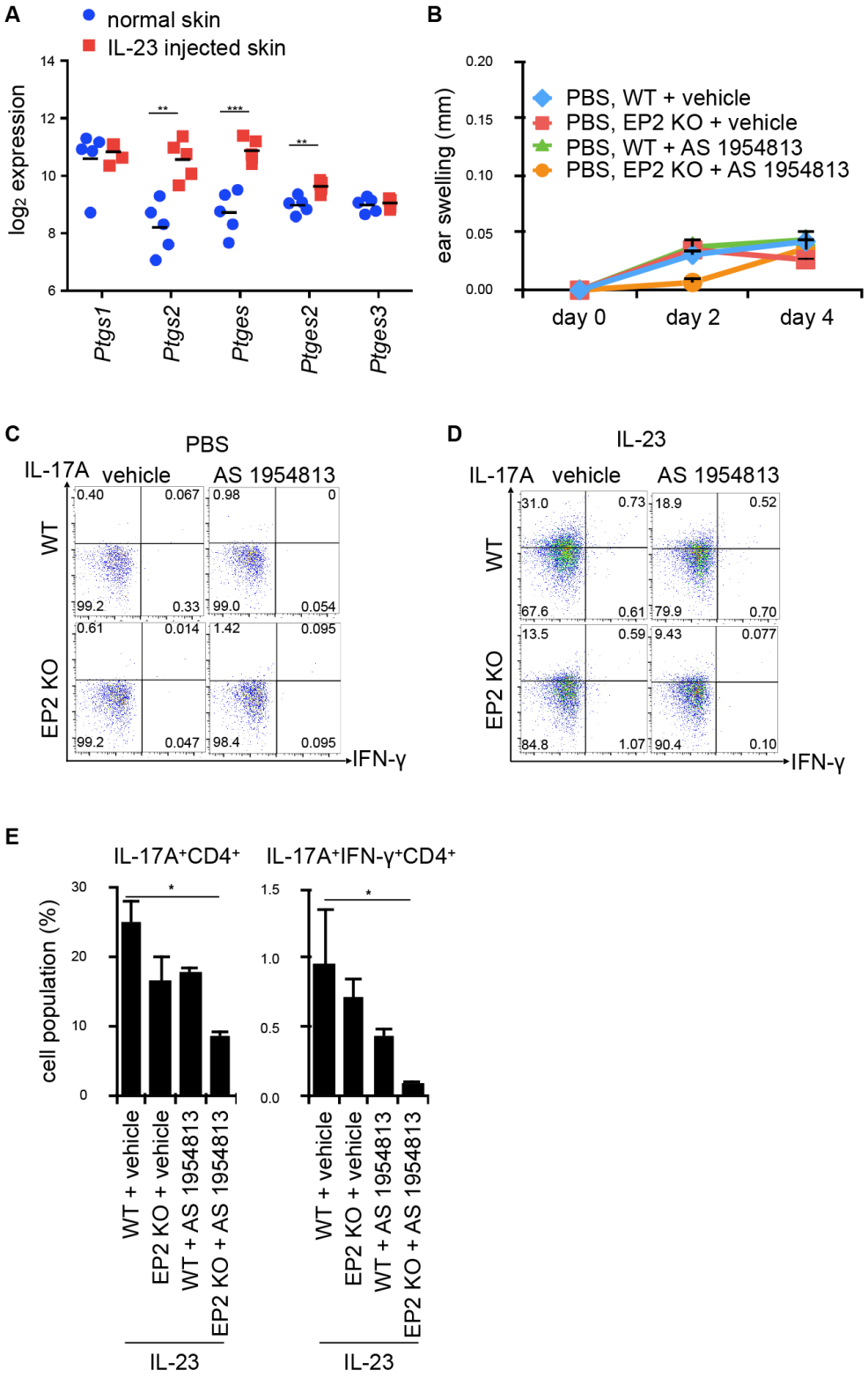
A
normal skin
IL-23 injected skin
log2 expression
Ptgs1
Ptgs2
Ptges
Ptges2
Ptges3
B
PBS, WT + vehicle
PBS, EP2 KO + vehicle
PBS, WT + AS 1954813
PBS, EP2 KO + AS 1954813
ear swelling (mm)
day 0
day 2
day 4
C
PBS
IL-17A
vehicle
AS 1954813
WT
EP2 KO
IFN-γ
D
IL-23
E
IL-17A+CD4+
IL-17A+IFN-γ+CD4+
cell population (%)
WT + vehicle
EP2 KO + vehicle
WT + AS 1954813
EP2 KO + AS 1954813
IL-23

Figure E4

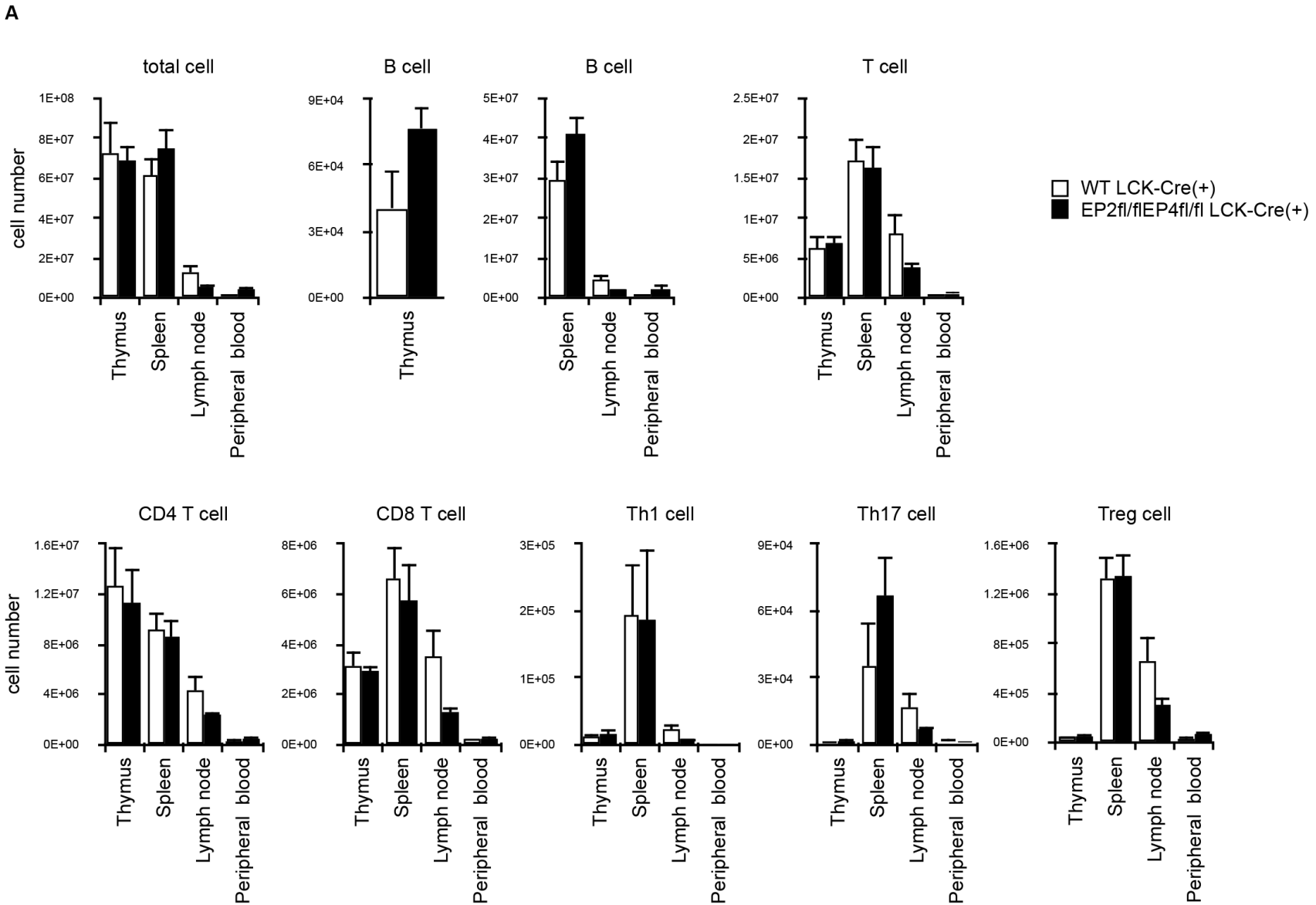
A
total cell
B cell
B cell
T cell
cell number
1E+08
8E+07
6E+07
4E+07
2E+07
0E+00
9E+04
6E+04
3E+04
5E+07
4E+07
3E+07
2E+07
1E+07
2.5E+07
2E+07
1.5E+07
1E+07
5E+06
Thymus
Spleen
Lymph node
Peripheral blood
WT LCK-Cre(+)
EP2fl/flEP4fl/fl LCK-Cre(+)
CD4 T cell
CD8 T cell
Th1 cell
Th17 cell
Treg cell
1.6E+07
1.2E+07
8E+06
4E+06
0E+00
6E+06
2E+06
3E+05
2E+05
1E+05
9E+04
6E+04
3E+04
1.6E+06
1.2E+06
8E+05
4E+05

Figure E5

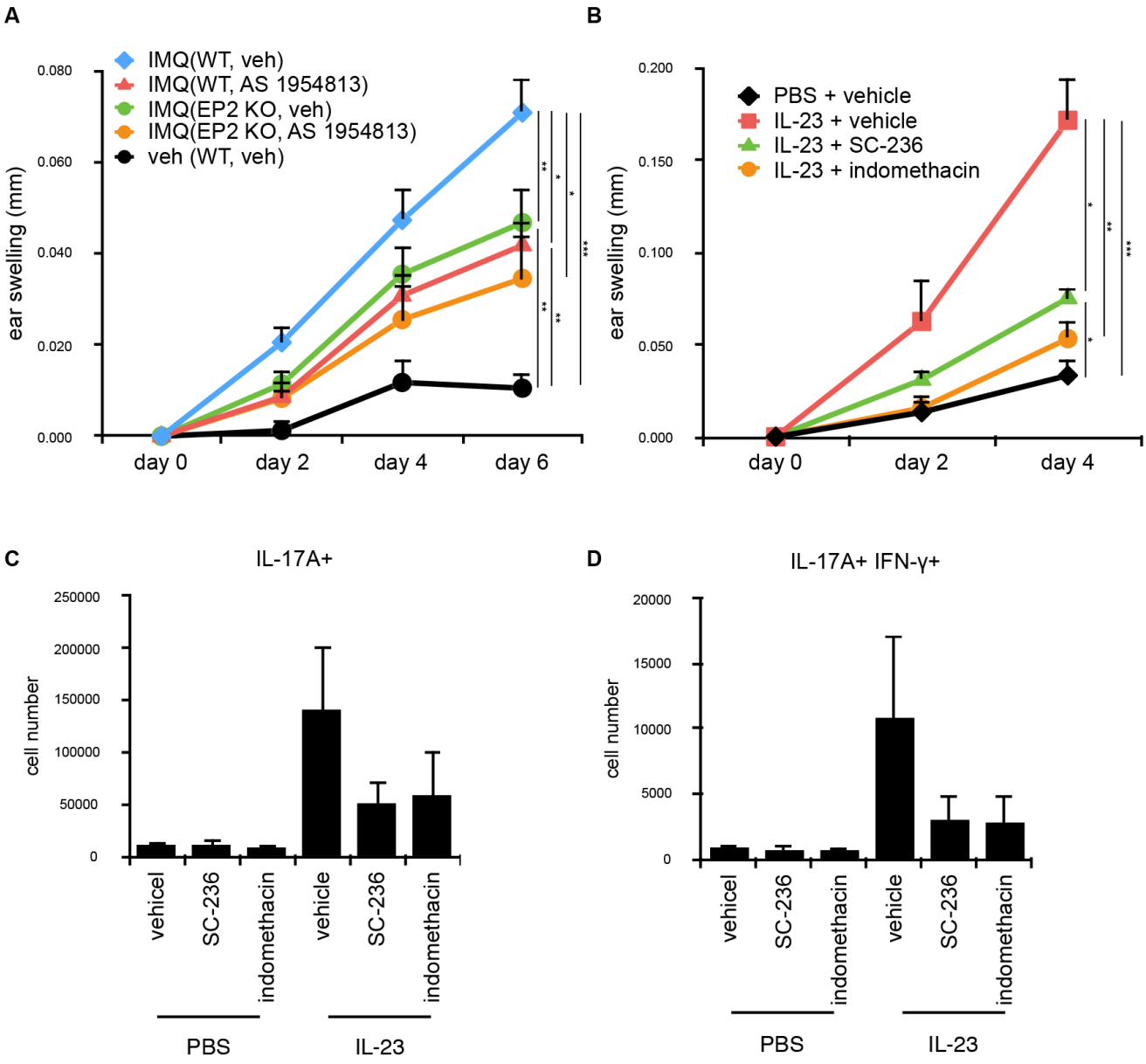
A
IMQ(WT, veh)
IMQ(WT, AS 1954813)
IMQ(EP2 KO, veh)
IMQ(EP2 KO, AS 1954813)
veh (WT, veh)
ear swelling (mm)
0.080
0.060
0.040
0.020
0.000
day 0
day 2
day 4
day 6
B
PBS + vehicle
IL-23 + vehicle
IL-23 + SC-236
IL-23 + indomethacin
ear swelling (mm)
0.200
0.150
0.100
0.050
0.000
day 0
day 2
day 4
C
IL-17A+
cell number
250000
200000
150000
100000
50000
0
vehicel
SC-236
indomethacin
vehicle
SC-236
indomethacin
PBS
IL-23
D
IL-17A+ IFN-γ+
cell number
20000
15000
10000
5000
0
vehicel
SC-236
indomethacin
vehicle
SC-236
indomethacin
PBS
IL-23

Figure E6

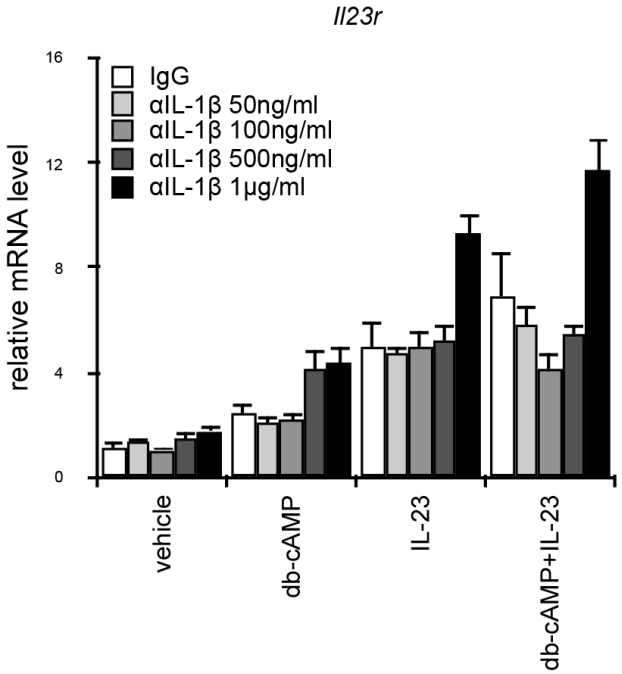
Il23r
relative mRNA level
16
12
8
4
0
IgG
αIL-1β 50ng/ml
αIL-1β 100ng/ml
αIL-1β 500ng/ml
αIL-1β 1μg/ml
vehicle
db-cAMP
IL-23
db-cAMP+IL-23

Figure E7