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STING signaling protects against chronic pancreatitis by modulating Th17 response

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Abstract

Objective: Chronic pancreatitis (CP) is an inflammatory disease with progressive fibrosis leading to exocrine and endocrine dysfunction. Currently, there are no approved effective therapies for CP. Stimulator of interferon genes (STING) signaling is a key innate immune sensor of DNA. In this study, we evaluated the role of STING signaling in CP.

Design: We used experimental model of CP to test the effect of STING signaling in STING wildtype (WT) and knockout (KO) mice as well as bone marrow chimeras (BMCs). STING was activated using a pharmacologic agent. Since we found changes in Th17 cells, we used neutralizing and control antibodies to determine the role of IL-17A. The effect of STING signaling was further explored in IL-17A generation and we examined the effect of IL-17A on pancreatic stellate cells (PSCs). Human pancreas from CP and non-CP patients were also stained for IL-17A.

Results: STING activation decreased CP associated pancreatic inflammation and fibrosis, whereas absence of STING led to worsening of the disease. BMCs showed that leukocytes play an important role in STING signaling mediated amelioration of experimental CP. STING deletion was associated with increased Th17 cell infiltration in the pancreas, whereas STING agonist limited this Th17 response. Importantly, anti-IL-17A antibody treatment mitigated the severity of CP in the absence of STING signaling. STING deficiency promoted Th17 polarization and PSCs express functional IL-17 receptor by upregulating fibrosis genes. Compared to tumor margins, pancreas from CP patients had significant increase in IL-17A^{+} cells.

Conclusion: Unlike acute pancreatitis, STING activation is protective in CP. STING signaling is important in regulating adaptive immune responses by diminishing generation of IL-17A during CP and presents a novel therapeutic target for CP.

Keywords

Chronic pancreatitis; STING; IL-17A

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Contributions

QZ, MM, and AH designed experiment, analyzed data and wrote manuscript; QZ, MM, and YW performed research; SJP provided key input; AH provided overall guide and supervision.

*Shared co-first authorship.

Conflict of Interest: None to declare for all authors regarding the work.

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Introduction

Chronic pancreatitis is described as progressive severe fibro-inflammatory condition with irreversible damage to the pancreas, characterized by acinar cell death, inflammation and fibrosis[1–3]. Currently, there are no FDA-approved therapies for chronic pancreatitis. Various animal models have been developed to understand the molecular mechanism and identify potential therapeutic targets for the disease[4]. Due to its chronicity and ongoing inflammation, exploring innate and adaptive immune signals during CP offers potential means of altering the natural course of the disease with hopes of rendering it from an "irreversible" to a reversible disease.

Stimulator of interferon genes (STING, encoded by TMEM173) signaling can sense abnormal DNA or cyclic dinucleotides in the cytosol of cells, as well as extracellular self DNA caused by apoptosis or necrosis, and bacterial or viral DNA[5–9]. STING signaling activation leads to induction of type I interferons (IFNs) and pro-inflammatory cytokines. Recently we showed that innate immune cells such as macrophages have the ability to sense DNA from dying acinar cells and STING activation promotes experimental acute pancreatitis[10]. Since recurrent acute pancreatitis can lead to chronic pancreatitis[11], we sought to understand the role of STING signaling during CP.

Here we investigated the role of STING signaling in a widely used experimental model of CP. Unlike in acute pancreatitis, we found that cGAS-STING signaling deficiency worsened chronic pancreatitis, whereas activation of STING signaling with a pharmacologic agent 5,6-dimethyllxanthenone-4-acetic acid (DMXAA) reduced severity of CP. Bone marrow chimera studies showed that STING signaling in leukocytes plays an important role in the observed protection against CP. Interestingly, absence of STING and its activation with DMXAA were associated with an increase and a decrease in Th17 cells in the pancreas respectively. Neutralization of IL-17A in the presence of STING deficiency mitigated severity of CP. Importantly, our ex-vivo studies show that STING activation restricts and its absence enhances Th17 polarization respectively. Moreover, we show that pancreatic stellate cells (PSCs) have functional IL-17 receptor and respond to IL-17A by activating ERK1/2 and upregulating fibrosis genes. In summary, our findings suggest that STING signaling plays an important role in protecting against CP by modulating Th17 response.

Material and methods

Mice

C57BL/6J mice, and C57BL/6J-Tmem173gt/J mice (STING KO mice) were purchased from Jackson Laboratories. cGAS KO mice were gifts from Dr. Lingyin Li (Stanford University, CA, USA). Animal care and use was approved by Stanford University institutional animal care and use committees.

Induction of chronic pancreatitis

Chronic pancreatitis was induced by repeated acute pancreatitis[12]. In brief, sex and age matched mice (6-8 weeks old) received 6 hourly intraperitoneal injection of 50μg/kg

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cerulein (Sigma-Aldrich, St Louis, MO) 3 days/week for a total of 4 weeks. Mice were sacrificed 3 days after the last cerulein injection as described[13]. For STING agonist treatment, mice were intraperitoneal injected with vehicle or 10mg/kg DMXAA (MedChem Express, New Jersey, USA) daily during the last 5 days of cerulein injection[14]. For antibody neutralizing experiments, mice were treated with either isotype control or anti-mouse IL-17A (anti-IL-17A, 50μg/mouse/day, 3 times/week; Bio X Cell, NH, USA) antibodies during the last 2 weeks[15]. To determine STING expression in leukocyte subsets (macrophages and Th17 cells) over time, mice receiving repeated cerulein or saline control (6 hourly injection/day, 3 days/week) were euthanized at week 1, 2, or 3.

Human pancreas tissues:

Human pancreas tissues from patients with CP and pancreatic cancer (where normal pancreas tumor margins were used) undergoing surgery were obtained from Stanford tissue bank with Local Ethics Committee approval and patient consents.

Histology, Immunohistochemistry (IHC), and Elastase1 Assay

Pancreas tissues were immediately taken from mice sacrificed by CO2 inhalation and fixed in 10% formalin. Fixed tissues were sent to Stanford Pathology Laboratory for processing of H&E and trichrome slides. Severity of fibrosis was quantified from trichrome staining and analyzed by Image J software (NIH, USA) following protocols as previously described[16, 17]. IHC assay was performed by Stanford Pathology Laboratory. IL-17A and CD45 (Abcam, Cambridge, United Kingdom) antibodies were used for the IHC assay. Tissue blocks from human CP (n=4) and non-CP (healthy tumor margin; n=4) pancreas were stained with IL-17A using IHC. Elastase1 was detected in pancreatic lysates from control and CP mice with pancreatic elastase 1 ELISA Kit (LSBio, Seattle, WA).

Cell cultures

Naive $CD4^+$ T cells were purified from spleen of wildtype or STING KO mice using mouse $CD4^+CD62L^+$ T Cell Isolation Kit II (Miltenyi Biotec, Bergisch Gladbach,Germany), then cultured in 1×10^6 cells/ml RPMI1640 containing 2 mM l-glutamine, 50 mM 2-ME, 100 U/ml penicillin, 100 mg/ml streptomycin, 10% fetal bovine serum (FBS) and 3mg/ml anti-CD28 (eBioscience, San Diego, CA) for 3 d in 96-well plates pre-coated with 1 μg/ml mouse anti-CD3 (eBioscience, San Diego, CA), and the polarizing cytokines: 5 ng/ml human IL-12 (Biolegend, San Diego, CA) and 10 ug/ml anti-mouse IL-4 (Biolegend, San Diego, CA) for Th1 differentiation; 5 ng/ml TGF-β (Biolegend, San Diego, CA), 10 ng/ml IL-6 (Biolegend, San Diego, CA), 10 μg/ml anti-IL-4 (Biolegend, San Diego, CA), 10 μg/ml anti-IFNγ (Biolegend, San Diego, CA) and 20 ng/ml IL-23 (Biolegend, San Diego, CA) for Th17 differentiation[18]. To test the effect of STING activation on Th17 polarization, 50ug/ml DMXAA was added to the culture on day 1 and the cells were collected for flow cytometry analysis on day 3[19, 20].

Mice PSCs were obtained from CP mice by outgrowth method and cultured in Dulbecco's modified Eagle medium/F12 containing 10% FBS[17]. To test the effect of IL-17A on PSCs, 100ng/ml recombinant mouse IL-17A protein (R&D system, Minneapolis, MN) was added

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at indicated time points[21]. In addition, in separate experiments supernatant from Th17 polarized cells in culture was added to the PSCs at indicated time points.

Bone marrow (BM) chimeric mice

BM chimeric mice were prepared as previously described[22]. C57BL/6J wild type (WT) recipient mice were irradiated with a dose of 9.5 Gy. Then each mouse received 5×10^6 BM cells from donor WT (WT→WT) or STING KO (KO→WT) mice by retro-orbital injection. Eight weeks later, the recipient mice WT→WT or KO→WT were subjected to cerulein-induced CP. In addition, WT and STING KO mice were irradiated with a dose of 9.5 Gy. Then each mouse received 5×10^6 BM cells from donor WT mice by retro-orbital injection. Eight weeks later, the recipient mice WT→WT or WT→KO were subjected to cerulein-induced CP.

Immunostaining for Flow cytometry

Isolation of pancreatic leukocytes was through collagenase digestion of mouse pancreas as previously described[13]. Dead cells were stained with LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit (Thermofisher scientific, Santa Clara, CA). For surface staining, cells were stained with antibody to the following markers: CD45, CD4, CD8, CD11b, F4/80, CD11c, CD44 and CD45RB (BioLegend, San Diego, CA). For intracellular staining, cells were stained with IFNγ, IL-4 and foxp3 (Biolegend, San Diego, CA), IL-17A and RORγt (eBioscience, San Diego, CA). Intracellular STING staining was as previously described[10], cells were stained with surface markers first, then fixed and permeabilized with a kit reagents from eBioscience (San Diego, CA). Then Rabbit unconjugated STING antibody or Rabbit IgG isotype control (Invitrogen, Carlsbad, CA) were used as primary antibody and AF488 conjugated goat anti-rabbit was used as secondary antibody (Life Technologies, Carlsbad, CA).

Quantitative Polymerase Chain Reaction (qPCR)

Mouse pancreas tissues were homogenized in TRIZOL and RNA was extracted as before[10]. Then cDNAs was reverse-transcribed from RNA with GoScript reverse transcription system (Promega, Madison, WI). qPCR was performed on ABI-7900 sequence detection system (Applied Biosystems, Foster City, CA) with primers and probes as previously described[10, 17]. Additional primers were as follow: mouse IL-6 forward, 5'-TCGGCAAACCTAGTGCGTTA-3'; mouse IL-6 reverse, 5'-CCAGCTGAGATGCGTCTTTC-3'; mouse MMP2 forward, 5'-CCAGACAGGTGACCTTGACC-3'; mouse MMP2 reverse, 5'-AAACAAGGCTTCATGGGGGC-3'; mouse MMP3 forward, 5'-CATCCCCTGATGTCCTCGTG-3'; mouse MMP3 reverse, 5'-CTTCTTCACGGTTGCAGGGA-3'. mouse b-actin forward, 5'-CGATGCCCTGAGGCTCTTTTCC-3'; mouse b-actin reverse 5'CATCCTGTCAGCAATGCCTGGG. The mRNA level was determined by normalizing to β-actin, and shown as fold change relative to control group.

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Western blot

Mouse pancreas tissues were homogenized in RIPA buffer (Cell Signaling, Danvers, MA) with protease inhibitor cocktail (Sigma, St. Louis, MO). Protein related to STING signaling was detected with antibodies as described previously[10]. ERK1/2, p-ERK1/2 (Cell Signaling, Danvers, MA), IL-17RA and αSMA (Abcam, Cambridge, United Kingdom) and actin (Santa Craz, CA) antibodies were used for Western blot.

Statistical Analysis

All statistical analyses were determined by Prism software (GraphPad Software Inc, La Jolla CA). The significance between two groups was determined by unpaired Student's t test. The differences among multiple groups were evaluated by one-way analysis of variance (ANOVA). A value of $P < 0.05$ was considered as statistically significant.

Results

STING activation is protective in CP

Since STING senses DNA from dying acinar cells and promotes acute pancreatitis[10], we hypothesized that STING signaling plays an important role in CP. To test this, we used cerulein-induced CP murine model, dependent on recurrent acute pancreatitis and widely used in the field. We induced CP in WT and STING KO mice. CP was more severe in STING KO as compared to WT mice as shown by pancreas weight, histology, PSCs activation and fibrosis related genes, such as *αSMA* (αSMA), *Fn1* (fibronectin 1) respectively (Figure 1A–C). At the same time, expression of STING downstream genes *IFNβ, Mx1* and *IRF7* were decreased (Figure 1D), indicating that lack of STING signaling worsens CP. Morever, leukocytes infiltration as shown by the pan-leukocyte marker (CD45) IHC staining was also increased in STING KO group (Figure 1E). As STING deficiency worsened CP, we examined STING associated pathways in cerulein-induced CP. STING and upstream sensor cGAS mRNA were increased significantly in pancreas of cerulein treated mice as compared to control saline treated mice (Figure 1F). In addition, STING protein and downstream STING signaling as shown by p-IRF3 increased significantly (Figure 1G). These results suggest that STING signaling is activated in the pancreas and plays a protective role in CP.

In CP, STING$^+$ CD4$^+$ T cells are increased and STING deficiency leads to an increase in Th17 cells in the pancreas

To better understand STING's role in CP, we first examined STING expression amongst pancreatic leukocytes. Consistent with Figure 1E and 1F findings, STING expression was increased in leukocytes during CP (Figure 2A). Within the STING$^+$ leukocyte (CD45$^+$) population the frequency of CD4$^+$ T cells increased (Figure 2B, C), whereas no changes were observed in CD8$^+$ T cells and macrophage frequencies (data not shown). We then went on to characterize the CD4$^+$ T cells subsets in CP mice. Compared to WT mice, IL-17A$^+$ T cells (Th17) increased while IFNγ$^+$ (Th1), IL-4$^+$ (Th2), and Foxp3$^+$ (Treg) cells did not increase in the pancreas of STING KO mice during CP (Figure 2D, E). Based on Ki67 staining, there was no significant change in IL-17A$^+$ T cell proliferation (Figure 2E). We did

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not see any difference between WT and STING KO mice IL-17A$^+$ T cells in the spleen during CP (data not shown). Morever, an increase in IL-17A$^+$ cells was observed by IHC staining in STING KO group (Figure 2F). These results indicate that STING deficiency promotes Th17 response in the pancreas during CP. Notably, pancreas from human CP also have significantly increased IL-17A$^+$ cells as compared to non-CP pancreas tissues (Supplementary Figure 1).

STING activation reduces CP and Th17 cells in the pancreas

Since the above studies suggested a protective role for STING, we used a STING agonist DMXAA [23, 24] to activate STING signaling during CP. In contrast to STING deficiency, STING activation with DMXAA reduced CP as shown by pancreas weight, histopathology, fibrosis score and fibrosis related gene expressions (Figure 3A–C). As expected, DMXAA increased STING downstream genes *IFNβ, Mx1* and *IRF7* expressions (Figure 3D). In contrast to the findings with STING KO mice, STING activation led to a decrease in IL1–7A$^+$ T cells in the pancreas of CP mice (Figure 3E). These results suggest that STING signaling provides a protection against CP by limiting Th17 response in the pancreas.

cGAS deficiency is associated with worse CP and an increase in Th17 cells in the pancreas

Cyclic GMP-AMP synthase (cGAS) is an upstream DNA sensor of STING. DNA stimulates cGAS to generate the second messenger 2’3’-cGAMP, which then binds and activates STING downstream signaling[25]. Thus, to evaluate the role of cGAS-STING signaling in CP, we examined the effect of cGAS deficiency in CP. Consistent with STING KO findings, CP was worse in cGAS KO mice as shown by pancreas weight, histopathology, fibrosis score, pancreatic elastase1 level (reflecting exocrine insufficiency), and fibrosis related gene expression (Figure 4A–C). IL-17A$^+$ but not IFNγ^+ CD4$^+$ T cells were increased in the pancreas in cGAS deficient mice during CP (Figure 4D). Taken together, these results show that cGAS-STING pathway is protective in CP and associated with a decrease in Th17 cells in the pancreas.

Leukocyte STING plays an important role in CP

Since STING is expressed in different cells types, we sought to determine the contribution of leukocyte STING in the CP protection observed. To this end, we generated chimeric mice by engrafting irradiated WT mice with either WT (WT-WT) or STING KO (KO-WT) bone marrow (BM) and induced CP (Figure 5A). Similar to STING KO mice, WT mice engrafted with STING KO BM (KO-WT) had more severe CP as compared to their WT BM recipients (WT-WT) as shown by histopathology, fibrosis score and fibrosis related genes (Figure 5B, C). Moreover, STING KO BM chimeras (KO-WT) had a significant increase in IL-17A$^+$ but not IFNγ^+ CD4$^+$ T cells in the pancreas (Figure 5D, E). In contrast, there was no difference in pancreas pathology or CP severity between WT chimeric mice engrafted with WT BM (WT-WT) and STING KO chimeric mice engrafted with WT BM (WT-KO) (Supplementary figure 2), suggesting that leukocyte but not non-leukocyte STING signaling plays a predominant protective role in CP.

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IL-17A neutralization improves CP

The studies above show that STING deficiency worsens CP and is associated with an increase in IL-17A$^+$CD4$^+$ T cells in the pancreas. To determine the significance of IL-17A, we treated WT and STING KO mice with either isotype or IL-17A neutralizing antibody during the last 2 weeks of the 4 weeks repetitive cerulein administration. In WT mice, there was no statistical difference in histopathology, fibrosis score and fibrosis related gene expression between mice treated with anti-IL-17A or isotype control antibody (Figure 6A–D). Whereas in STING KO mice, IL-17A neutralization led to significant improvement in CP severity as shown by pancreas weight, histopathology, fibrosis score and gene expression (Figure 6A–D). These studies suggest that IL-17A in part mediates the CP severity observed in STING KO.

STING activation inhibits Th17 polarization and IL-17A promotes fibrosis gene expression

To test whether STING directly impacts IL-17A generation, we set up in vitro T cell polarization assay using naive CD4$^+$ T cells isolated from WT and STING KO mice spleens. Like the in vivo findings, STING deficiency promoted Th17 but not Th1 (Figure 7A, B) or Th2 (not shown) polarization. In contrast to STING deficiency, STING activation with DMXAA in WT CD4$^+$ T cells markedly inhibited IL-17A production (Figure 7C). These findings suggest that STING signaling has T cell intrinsic effect.

Since IL-17A neutralization in STING KO mice improved fibrosis (Figure 6), we examined whether PSCs express IL-17 receptor and respond to IL-17A. As reported for hepatic stellate cells[20], we found that PSCs express IL-17RA and respond to IL-17A by activating downstream ERK/1/2 (Figure 7D) and upregulating fibrosis genes (Figure 7E), IL-6, and matrix metalloproteinases (MMPs) (Supplementary figure 3A). Morever, PSCs respond to recombinant IL-17A and also to Th17 cell conditioned medium by activating downstream ERK/1/2 (Supplementary figure 3B). Taken together the above results suggest that in the absence of STING signaling, Th17 polarization is enhanced and IL-17A promotes PSC fibrosis gene expression.

Discussion

STING activation or DNA sensing is important for normal host defense against pathogens, but can have deleterious effects when self DNA triggers inflammation in disease states such systemic lupus erythematosis[26] . We also recently showed that STING activation worsens acute pancreatitis severity in experimental models via macrophage sensing of DNA released from dying acinar cells[10]. In this study, we find that STING signaling during CP alters the adaptive immune response by regulating Th17 generation. Unlike in acute pancreatitis, STING activation plays a protective role in CP by regulating Th17 response.

Our previous study showed that STING signaling is activated in macrophages and promotes TNFα and IFNβ release and worsens acute pancreatitis[10]. Here we found that macrophages are also the main subset of STING$^+$ leukocytes, however their frequency (STING$^+$ macrophages) amongst total CD45$^+$ leukocytes were not different between cerulein (CP) and saline control treated mice (data not shown). In contrast, among pancreas STING$^+$

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leukocytes, $CD4^+$ T cells were increased in CP mice. This led us to further examine $CD4^+$ T cell subsets in the injured pancreas and found that STING signaling deficiency leads to an increase in pancreas Th17 cells and worsens pancreatic fibrosis. In this CP model, as $STING^+$ macrophages start to decrease, $STING^+$ $IL\text{-}17A^+CD4^+$ T cells start to increase mid point (or at 2 weeks) of the repeated 3 weeks cerulein administration, suggesting a transition period in which there is progression towards CP that is associated with rise of $STING^+$ Th17 cells (Supplementary figure 4). Thus, STING signaling regulates different immune cell subsets under different pathologic or during acute versus chronic inflammatory conditions.

STING activation inhibits T cell proliferation in human cells and STING deficiency can promote T cell proliferation in mice[27], but the effect of STING signaling in T cell differentiation remains unknown. Here we found that absence of STING promotes Th17 cell increase in the pancreas during CP, but no changes were observed in Th1, Th2, or Tregs. In vitro T cell polarization studies confirmed this finding where absence of STING promoted Th17 polarization while activation of STING markedly inhibited Th17 cell generation. Thus cGAS-STING signaling can directly modulate Th17 polarization and the specific molecular mechanism is an interest for future investigation (Figure 7F).

Similar to the CP model, we found that $IL\text{-}17A^+$ cells were increased in human CP tissues as compared with non-CP control tissues. In our cerulein induced CP model, anti-IL-17A treatment reduced severity of CP in STING KO group, but did not have as much effect in the STING WT CP group, perhaps due to the higher extent of IL-17A overexpression observed in STING KO mice. In addition, consideration need to be taken into account that other drivers might also be contributing that are independent of STING signaling. IL-17A was reported to activate hepatic PSCs cells and promote liver fibrosis[21, 28]. Our findings parallel these findings, and to our knowledge there are no reports whether PSCs express IL-17RA. Herein, we found that PSCs express functional IL-17 receptor, and IL-17A induces PSCs to upregulate fibrosis gene expression, indicating that IL-17A may promote fibrosis in different chronic diseases. It is therefore conceivable to propose blockade of this pathway to limit and/or slow down fibrosis progression in different organs.

IL-17A overexpression in the pancreas promotes PanIN initiation and progression, while IL-17A deletion in hemtopoietic cells delays this progression[29]. Thus, the link between STING signaling and IL-17A we made in this study might also be relevant in pancreas cancer. Infact, STING signaling is reported to play an important role in anti-tumor response[19, 30–32], and STING agonist treatments inhibit tumor progression in various models[33]. These studies utilized various approaches to activate STING and enhance efficiency of STING agonist, which included nanosatellite vaccine and cationic silica nanoparticles [34, 35]. DMXAA can bind mouse STING [36], and functionally activate STING leading to downstream TBK1 and IRF3 signaling and induce type I IFNs[37, 38]. In our study, we used this widely available and well-studied STING agonist DMXAA, and show that DMXAA is effective in treating established experimental CP. Thus STING agonist developed for clinical use are promising therapeutic targets for CP. Taken together, our study reveal that cGAS-STING signaling is protective in CP via regulating Th17 response, and we propose this pathway as a novel target for the treatment of CP.

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Supplementary Material

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Abbreviations:

STING	Stimulator of interferon genes
WT	wildtype
KO	knockout
BMCs	bone marrow chimeras
αSMA	α-smooth muscle actin
DMXAA	5,6 dimethyllxanthenone-4-acetic acid
BM	bone marrow
CP	chronic pancreatitis
ECM	extracellular matrix
IL	interleukin
PSC	pancreatic stellate cell
ERK	extracellular signal-regulated kinases
TEM	T effector/memory
IFNs	interferons
Fn1	fibronectin1

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Summary: Significance of this study

What is already known about this subject?

(1) Recurrent acute pancreatitis can lead to chronic pancreatitis and there are currently no active therapies for chronic pancreatitis. (2) STING activation worsens acute pancreatitis, but its role in chronic pancreatitis is not known. (3) Macrophages have the ability to sense pancreatic acinar cell death and produce pro-inflammatory cytokines.

What are the new findings?

(1) Unlike in acute pancreatitis, we found that STING signaling is protective in chronic pancreatitis and limits fibrosis. (2) We found this protection to be associated with alteration in adaptive immunity with a decrease in IL-17A$^+$ cells in the pancreas. (3) STING deficiency-mediated worsening of chronic pancreatitis could be reversed with IL-17A neutralization. (4) STING deficiency leads to augmented Th17 polarization, whereas STING activation restricts Th17 generation. (5) Pancreatic stellate cells express functional IL-17 receptor and respond to IL-17A by activating ERK1/2 and upregulating fibrosis genes.

How might it impact on clinical practice in the foreseeable future?

We found that STING signaling is important in regulating adaptive immune responses and limiting inflammation during chronic pancreatitis. IL-17A$^+$ cells are increased in human CP tissues. Activation of STING using a pharmacologic agent reduces experimental chronic pancreatitis and this provides a novel therapeutic target.

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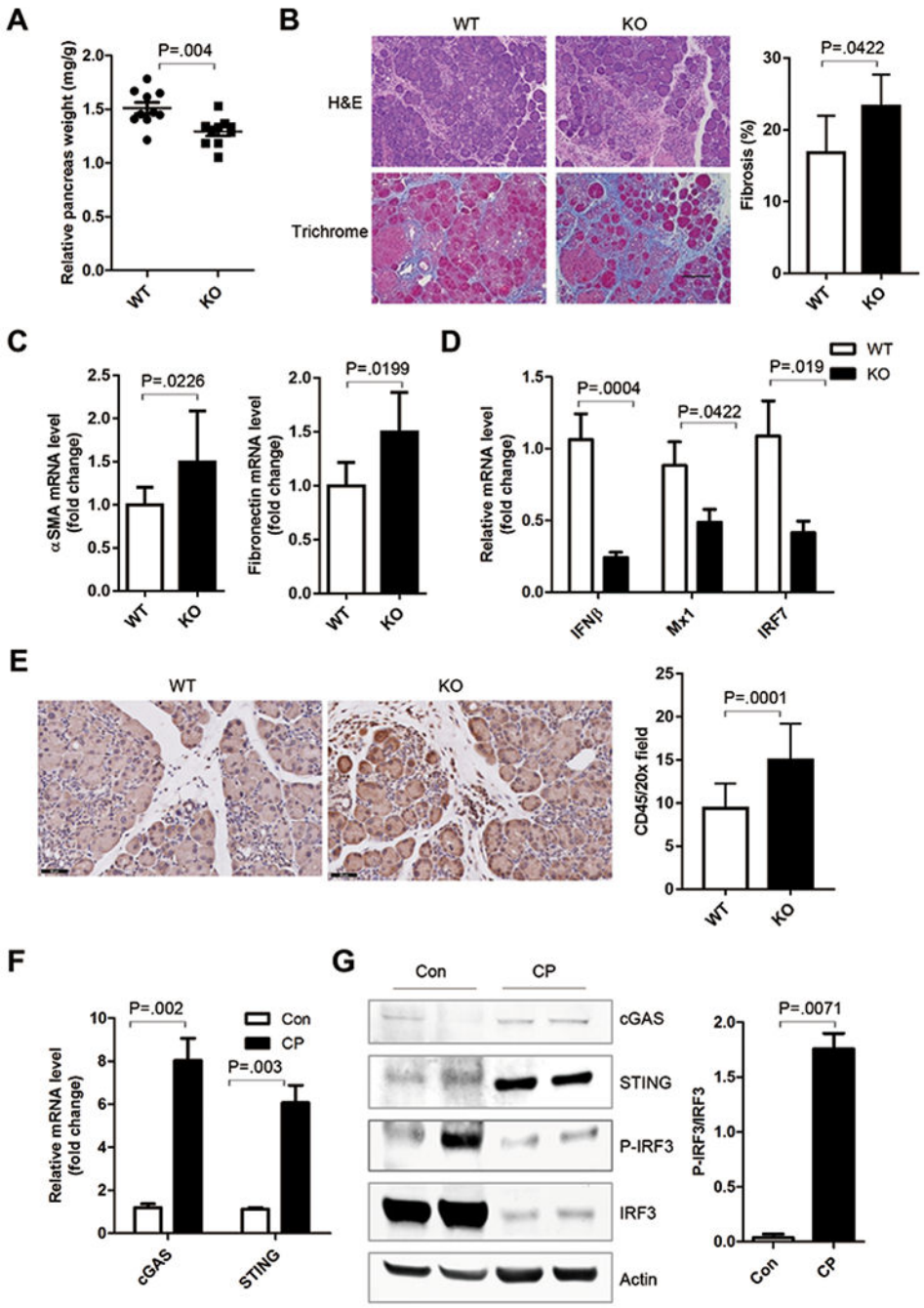

Figure 1. STING signaling is protective in CP

(A) Relative pancreas weight of WT and STING KO CP mice. (B) Representative of pancreas H&E and trichrome staining. Scale bar=100 μm. Bar graph shows quantitation of fibrosis (mean ± SD). (C, D) qPCR analysis of *αSMA* (αSMA), *Fn1* (fibronectin), and STING downstream signaling in the pancreas. (n =10 for all groups, mean ± SD). (E) Representative of pancreas sections stained with pan-leukocyte marker. Scale bar=50 μm. Bar graph shows $CD45^+$ infiltrating cells in 20x field (mean ± SD). (F) *cGAS* and *STING* expression by qPCR in pancreas during CP. CP, chronic pancreatitis; Con, control saline

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treated mice, Data presented as mean ± SD from 3 independent experiments (n = 4 mice per group and per experiment). (G) Pancreas cGAS, STING, and downstream proteins were determined by western blot.

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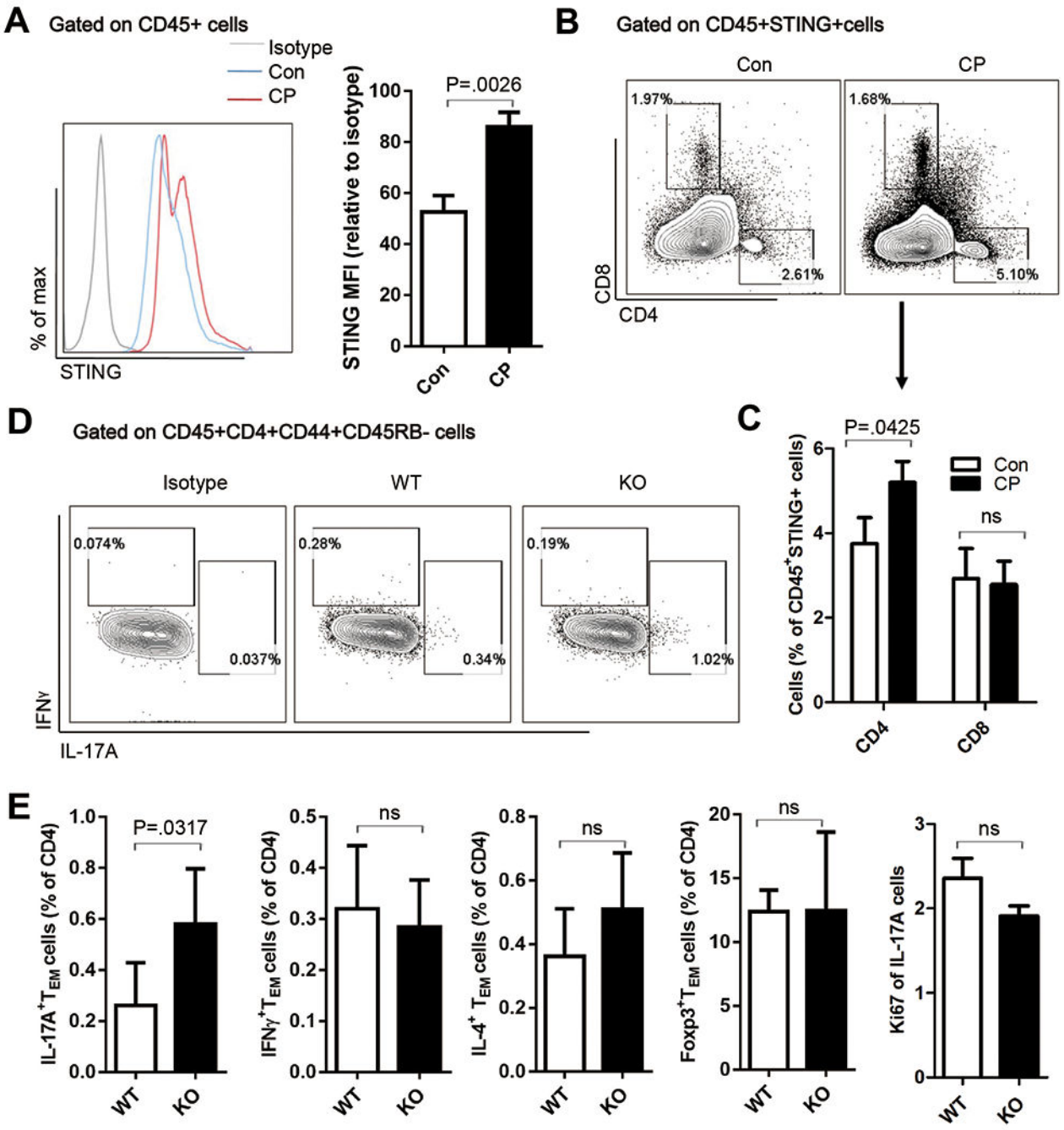

Figure 2. CD4$^+$ T cells increase in STING$^+$ leukocytes and STING deficiency leads to an increase in Th17 cells

(A) Pancreas leukocytes were isolated for flow cytometry and STING expression in live leukocytes (CD45) is shown. (B, C) STING$^+$ leukocyte subsets in control and CP mice. (D) Representative plot of pancreas leukocytes analyzed by flow cytometry. (E) Bar graph representing T cell groups analyzed by flow cytometry is shown. CD4$^+$ T_{EM} represents T effector/memory cell population (CD45$^+$CD4$^+$ CD44^{hi}CD45RB^{low}). (n =10 for all groups, mean ± SD). (F) Representative of pancreas sections stained with IL-17A. Scale bar=50 μm. Bar graph shows IL-17A$^+$ infiltrating cells in 20x field (n=5, mean ± SD).

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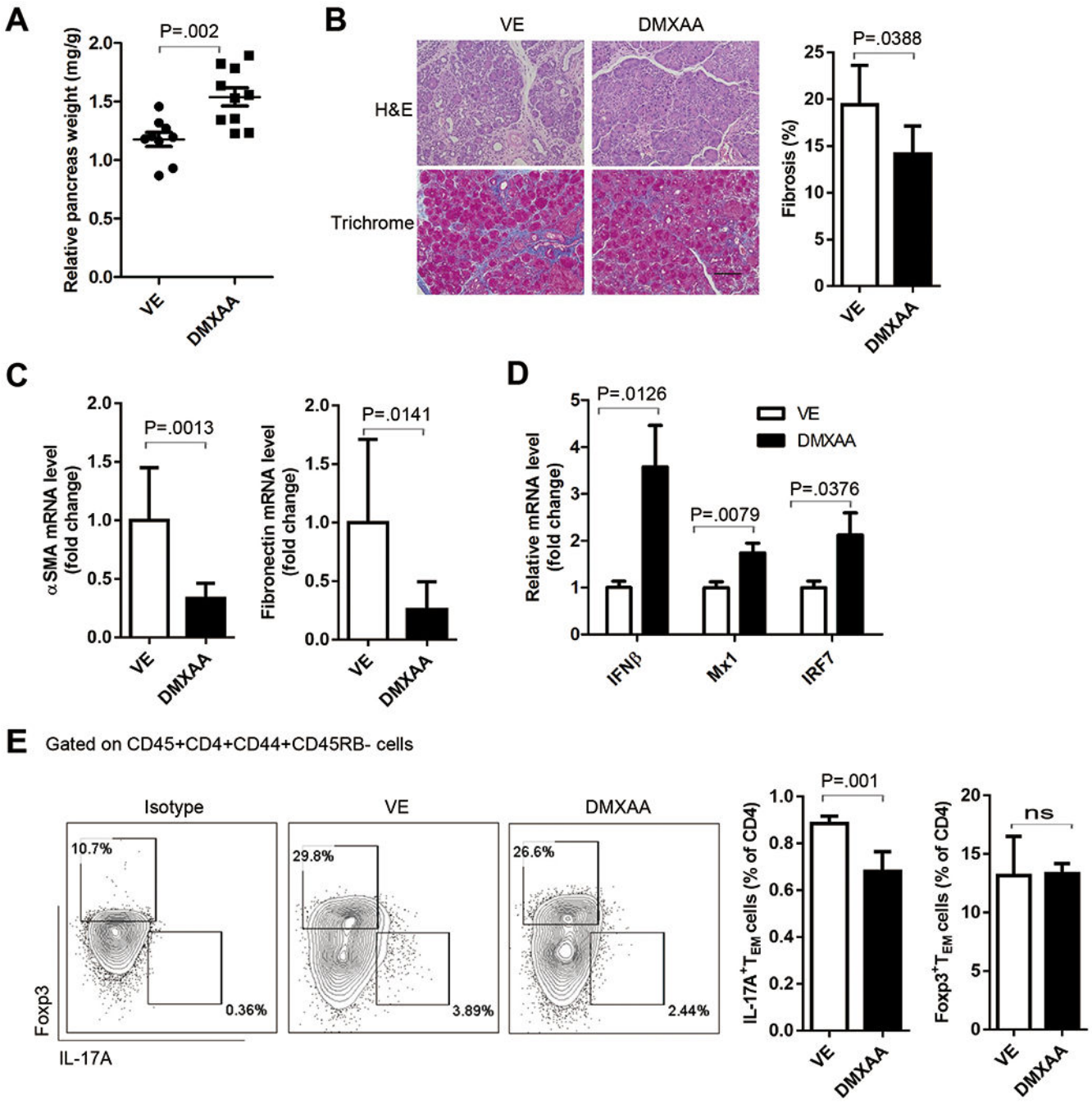

Figure 3. STING activation reduces CP and Th17 cells in the pancreas

(A) STING activation using DMXAA treatment of C57BL/6J mice with CP. DMXAA (10mg/kg) or vehicle control (VE) were administered i.p. daily during the last 5 days of cerulein injection. Relative pancreas weight for the groups is shown. (B) Representative of pancreas H&E and trichrome staining. Scale bar=100 μm. Bar graph shows quantitation of fibrosis (mean ± SD). (C, D) qPCR analysis of *αSMA* (αSMA), *Fn1* (fibronectin), and STING downstream signaling in the pancreas. (E) Representative of pancreas leukocyte analyzed by flow cytometry. (F) Bar graph representing T cell groups analyzed by flow

cytometry. $CD4^+$ T_{EM} represents T effector/memory cell population ($CD45^+CD4^+CD44^{hi}CD45RB^{low}$). (A-F) For all experiments n=10, 3 independent experiments, mean ± SD.

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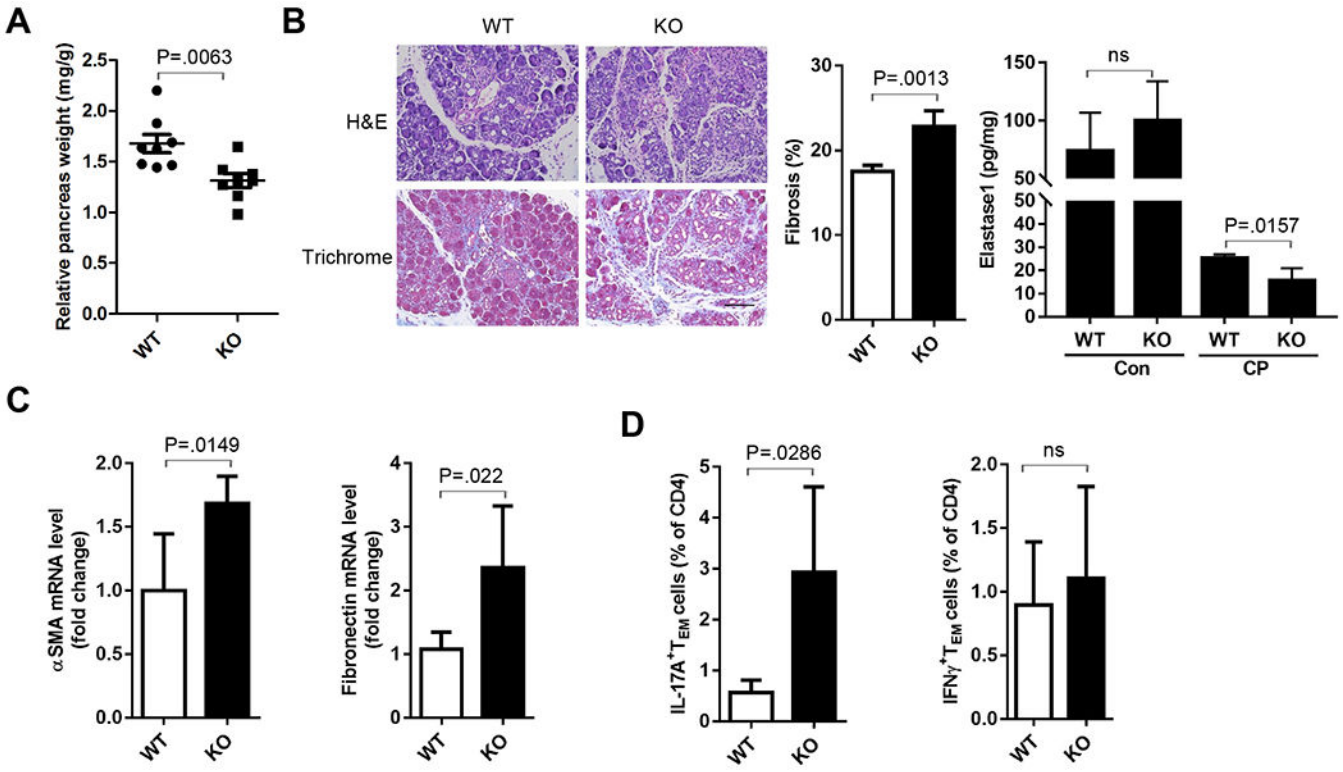

Figure 4. CP is worse in cGAS KO mice and associated with an increase in Th17 cells in the pancreas

(A) Relative pancreas weight of WT and cGAS KO CP mice. (B) Representative of pancreas H&E and trichrome staining. Scale bar=100 μm. Bar graph shows quantitation of fibrosis and elastase1 level in pancreas of control (saline treated) and CP mice (mean ± SD). (C) qPCR analysis of *αSMA* (αSMA) and *Fn1* (fibronectin) in the pancreas. (D) Bar graph representing T cell groups analyzed by flow cytometry. $CD4^+$ T_{EM} represents T effector/memory cell population ($CD45^+CD4^+$ $CD44^{hi}CD45RB^{low}$). (A-D) For all experiments, n =8, 3 independent experiments, mean ± SD.

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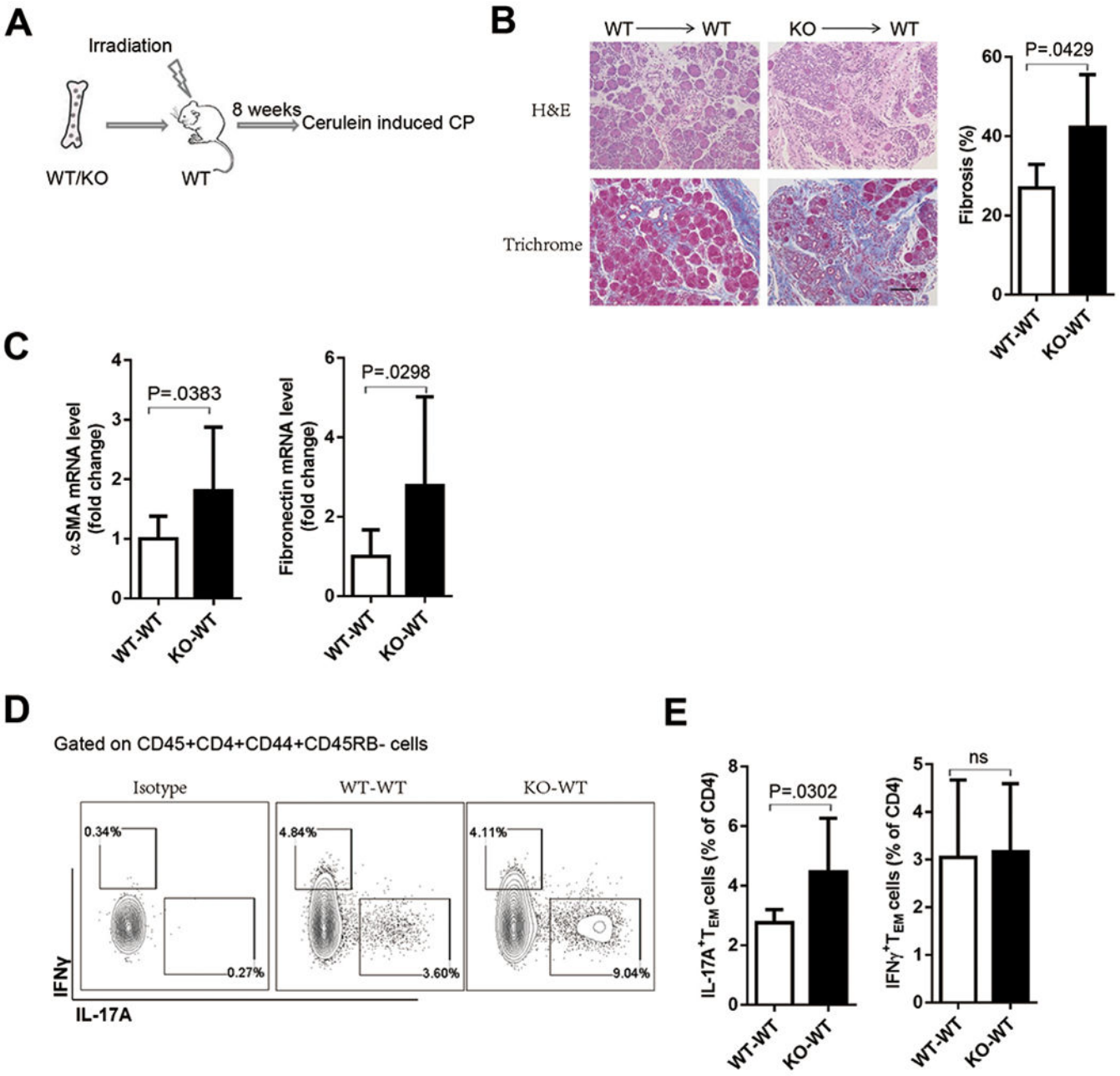

Figure 5. Leukocyte STING plays an important role in CP

(A) Irradiated WT mice were transplanted with WT or STING KO bone marrow to generate WT-WT and STING KO-WT bone marrow chimeras respectively. CP was induced after 8 weeks of bone marrow engraftment using repetitive cerulein injection. (B) Representative of pancreas H&E and trichrome staining. Scale bar=100 μm. Bar graph shows quantitation of fibrosis (mean ± SD). (C) qPCR analysis of *αSMA* (αSMA) and *Fn1* (fibronectin) in the pancreas. (D) Representative of pancreas leukocyte analyzed by flow cytometry. (E) Bar graph representing T cell groups analyzed by flow cytometry. $CD4^+$ T_{EM} represents T effector/memory cell population ($CD45^+CD4^+$ $CD44^{hi}CD45RB^{low}$). (A-E) For all experiments, n =10, at least 2 independent experiments, mean ± SD.

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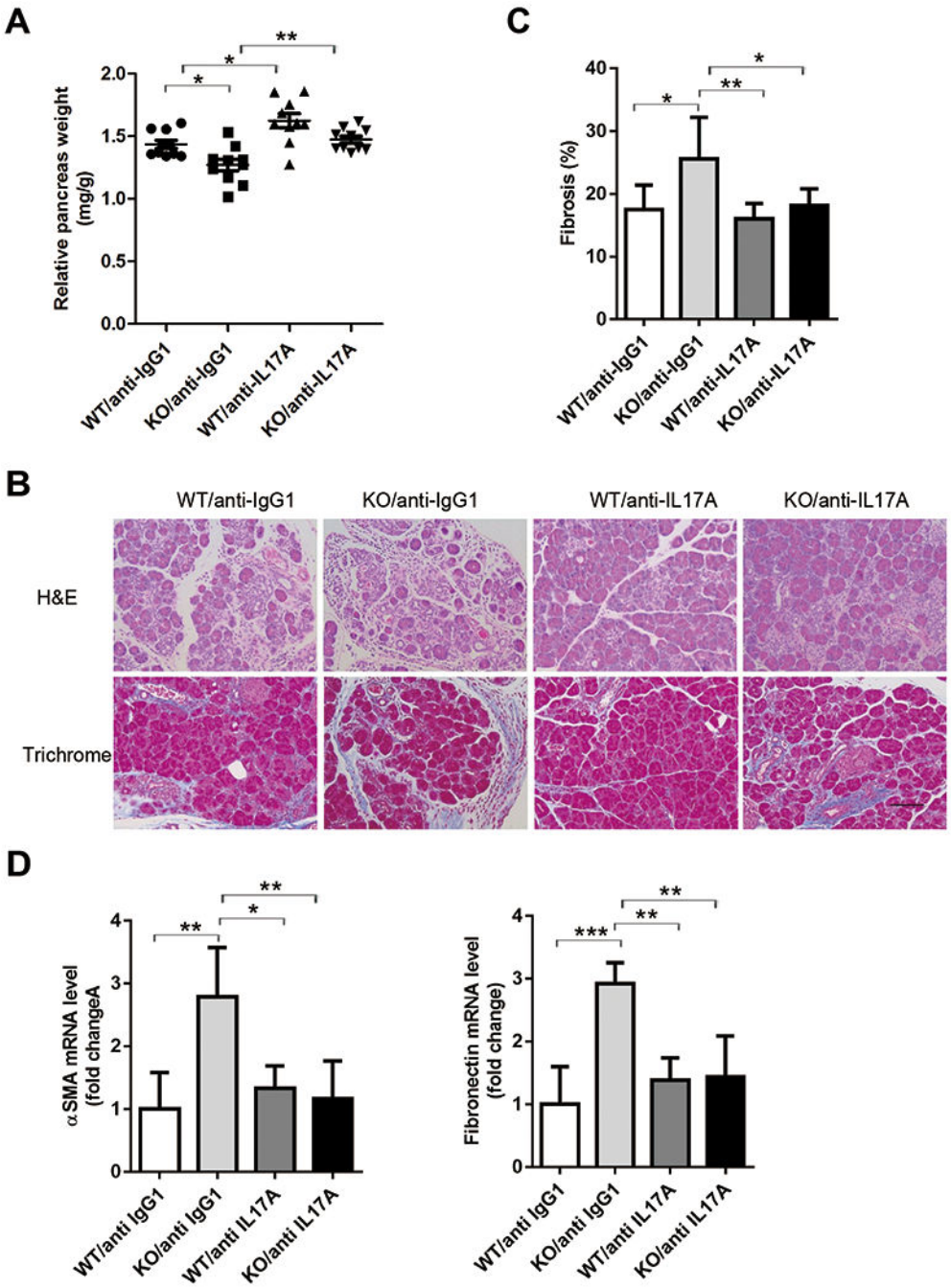

Figure 6. IL-17A neutralization improves CP

(A) Relative pancreas weight of WT and STING KO CP mice treated with either isotype (anti-IgG1) or anti-IL-17A antibody. (B, C) Representative of pancreas H&E and trichrome staining. Scale bar=100 μm. Bar graph shows quantitation of fibrosis (mean ± SD). (D) qPCR analysis of *αSMA* (αSMA) and *Fn1* (fibronectin) in the pancreas. (A-D) For all experiments, n=10, at least 2 independent experiments, mean ± SD.

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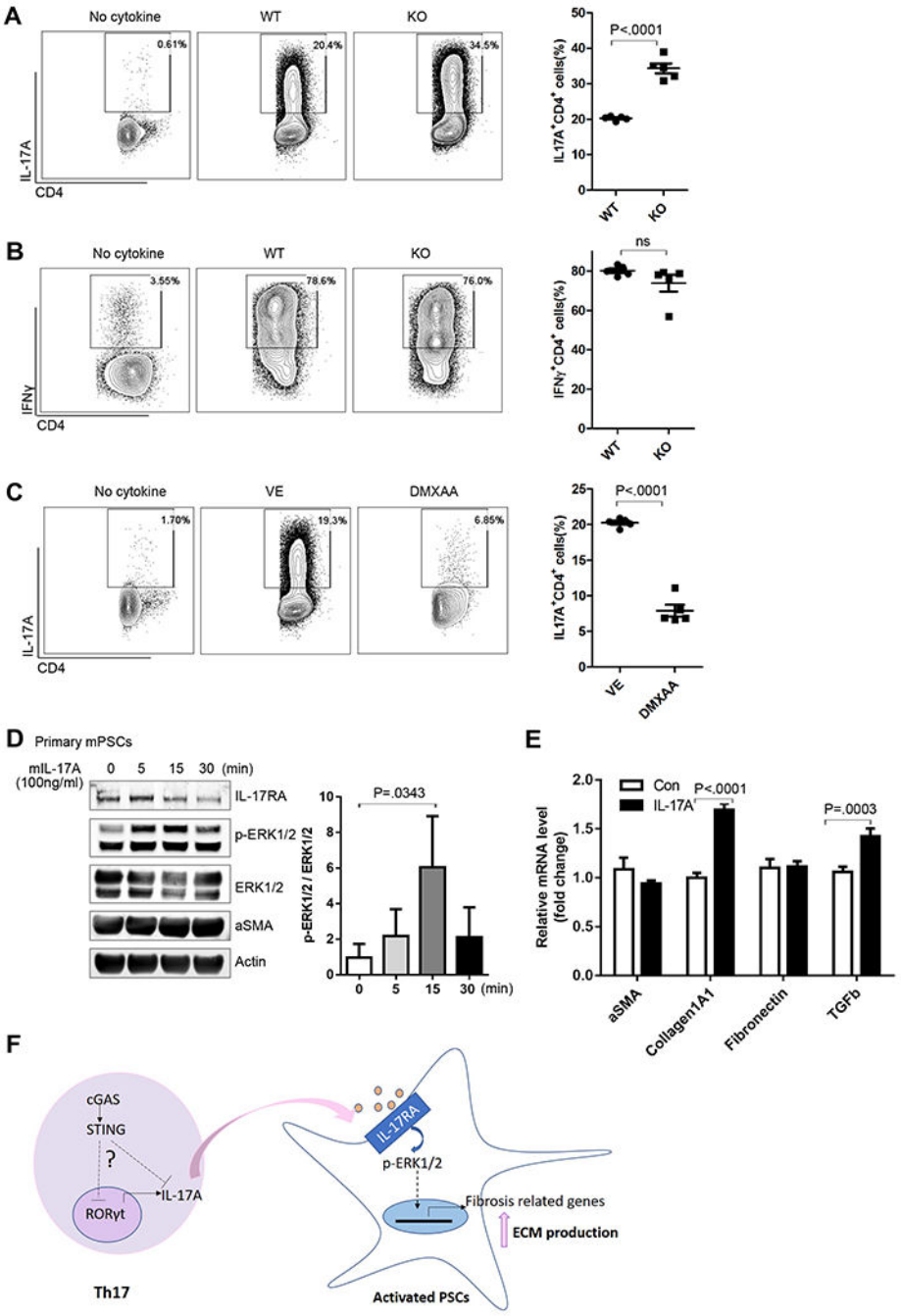

Figure 7. Absence of STING promotes Th17 polarization and IL-17A promotes fibrosis gene expression in vitro

(A, B) Naive $CD4^+$ T cells from spleen of WT and STING KO mice were differentiated into Th17 cells or Th1 cells. On day 3, the cells were collected and stimulated with PMA, ionomycin and Brefeldin A for 5 h. The frequencies of IL-$17A^+$ (Th17) and IFNγ^+ (Th1) cells were examined by flow cytometry. (C) 24h after the naive $CD4^+$ T cells from spleen of WT mice were cultured for differentiation into Th17 cells, VE control or 50ug/ml DMXAA was added to the cell culture supernatant. On day 3, the cells were collected and stimulated with PMA, ionomycin, Brefeldin A for 5 h. The frequencies of IL-$17A^+$ cells were

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examined by flow cytometry. Error bars show mean ± SD, n = 5 in each group. Data representative from three experiments are shown. (D) Primary mouse pancreatic stellate cells (mPSCs) isolated from WT CP mice were treated with mouse IL-17A (100 ng/mL) for indicated times and then lysed for western blot with p-ERK1/2, ERK1/2, IL-17RA, and αSMA. Data shown is from a representative of 3 independent experiments. Relative p-ERK1/2 expression (p-ERK1/2 / ERK1/2) is shown as bar graph (mean ± SD, one-way ANOVA). (E) mPSCs were treated with VE or mouse IL-17A (100 ng/mL) for 24 hours. mRNA expression of *αSMA* (αSMA), *Fn1* (fibronectin), collagen1A1 and *TGFβ* (TGFb) were detected by qPCR. Bar graph represents mean ± SD (n=3 independent experiments). (F) Schematic presentation of Th17 and pancreatic stellate cell (PSC) interaction during CP.