A defect in COPI-mediated transport of STING causes immune dysregulation in COPA syndrome

**Introduction**

COPA syndrome is a genetic disorder of immune dysregulation caused by missense mutations that disrupt the WD40 domain of coatamer protein complex subunit a (COPA; Watkin et al., 2015). COPA is a subunit of coat protein complex I (COPI) that mediates retrograde movement of proteins from the Golgi apparatus to the ER (Adolf et al., 2019). Prior studies have shown that alterations to the COPA WD40 domain lead to impaired binding and sorting of proteins bearing a C-terminal dilysine motif as well as a defect in retrograde Golgi to ER transport (Eugster et al., 2000; Watkin et al., 2015). To date, the molecular mechanisms of COPA syndrome remain unknown, including whether missorted proteins are critical for initiating the disease.

A clue to the pathogenesis of COPA syndrome recently arose with the observation that type I interferon signaling appears to be highly dysregulated in the disease (Volpi et al., 2018). This led us to investigate whether COPA syndrome shares features with any of the well-described Mendelian interferonopathy disorders (Uggetti et al., 2019). COPA syndrome manifests similarly to the type I interferonopathy STING-associated vasculopathy with onset in infancy (SAVI). Both diseases present at an early age with interstitial lung disease, activation of type I interferon-stimulated genes (ISGs), and evidence of capillaritis (Tsui et al., 2018; Liu et al., 2014). Stimulator of interferon genes (STING) is an ER-localized transmembrane protein involved in innate immune responses to cytosolic nucleic acids. After binding cyclic dinucleotides, STING becomes activated as it translocates to the ER-Golgi intermediate compartment and Golgi. At the ER-Golgi intermediate compartment/Golgi, STING forms multimers and activates the kinase TBK1 which subsequently phosphorylates the transcription factor IRF3 to induce expression of type I interferons and other cytokines (Gui et al., 2019). In SAWI, gain-of-function mutations cause STING to aberrantly exit the ER and traffic to the Golgi and become activated (Dobbs et al., 2015). Prior work has suggested that COPI may be involved in STING transport at the Golgi, but this is not well established, and the molecular interactions between COPI and STING remain unknown (Gui et al., 2019; Ablasser and Hur, 2020). Because COPA plays a critical role in mediating Golgi to ER transport, we hypothesized that activation of type I interferon signaling in COPA syndrome involves missorting of STING.

**Results and discussion**

To examine this, we assessed lung fibroblasts from a COPA syndrome patient to determine if there was evidence of STING
activation. We measured mRNA transcript levels of several ISGs and found they were significantly elevated in comparison to healthy control lung fibroblasts in the presence or absence of a STING agonist (Fig. 1 A and Fig. S1, A and B). Confocal microscopy of COPA syndrome fibroblasts revealed prominent colocalization of STING with the Golgi (Fig. 1 B). Western blots of cellular protein lysates showed an increase in STING multimerization (Fig. 1 C) consistent with localization of STING at the Golgi and also higher levels of phosphorylated TBK1 (pTBK1) and phosphorylated STING (Fig. 1 D and Fig. S1 C), indicative of STING activation (Srikanth et al., 2019). These data suggest that the elevated type I ISGs observed in COPA syndrome patients may be caused by spontaneous activation of STING.

To establish the specific role of mutant COPA in triggering STING pathway activation, we transduced cells with retroviral vectors encoding enhanced GFP (EGFP)–STING and then transfected them with plasmids encoding wild-type or E241K mutant COPA. We performed confocal microscopy to examine whether mutant COPA caused STING to localize on the Golgi, similar to what we observed in patient fibroblasts. In cells expressing wild-type COPA, EGFP-STING was normally distributed throughout the cytoplasm (Gui et al., 2019), whereas in cells with E241K mutant COPA, EGFP-STING colocalized with the Golgi marker GM130 (Fig. 2 A). We next reconstituted human embryonic kidney 293 cell line (HEK293)T cells (which lack endogenous STING) with retroviral vectors encoding EGFP-STING and then transfected cells with wild-type or E241K mutant COPA. We found that even in the absence of a STING agonist, cells demonstrated a significant increase in pTBK1 (Fig. 2 B) and higher levels of mRNA transcripts encoding IFNB1 and type I ISGs (Fig. 2 C). Importantly, all of these increases were abolished in HEK293T cells without EGFP-STING, indicating that mutant COPA activates TBK1 and type I interferon signaling specifically through STING rather than other innate immune pathways such as TIR-domain–containing adapter–inducing interferon β or mitochondrial antiviral signaling protein (Fig. 2, B and C; Liu et al., 2015). Taken together, these data show that mutant COPA causes ligand-independent activation of STING on the Golgi with up-regulation of type I interferon signaling.

We hypothesized that retention of STING on the Golgi might reflect a failure of STING to be taken up into mutant COPA containing COPII complexes for transport to the ER. To evaluate
this, we analyzed the protein–protein interaction between COPA and STING. We performed coimmunoprecipitation experiments and found that although we could pull down STING with wild-type COPA, the amount of STING that coimmunoprecipitated with E241K mutant COPA was substantially reduced (Fig. 2 D). We previously showed that disease-causative COPA mutations cause a defect in binding between the COPA WD40 domain and C-terminal dilysine motif (e.g., KKxx and KxKxx) of proteins targeted for retrieval to the ER (Watkin et al., 2015). Because STING lacks a dilysine tag, we hypothesized that an adaptor protein mediates the interaction between STING and COPA. A review of published studies uncovered seven STING interacting partners that contain a C-terminal dilysine motif (Fig. S2 A; Lee et al., 2013; Huttlin et al., 2017; Shang et al., 2018; Li et al., 2011).
Among these, SURF4 was the only protein shown to cycle between the ER and the Golgi via COPI (Mitrovic et al., 2008; Adolf et al., 2019) and function as a cargo receptor (Emmer et al., 2018). Thus, we speculated that SURF4 was a likely candidate for mediating an interaction between COPA and STING. We confirmed through coimmunoprecipitation assays that SURF4 associates with STING and COPA (Fig. 2 E and Fig. S2 B) and then mutated the C-terminal dilysine motif of SURF4 by replacing the lysines at positions -3, -4, and -5 from the C terminus with serines (SURF4-SSS). The amount of SURF4-SSS pulled down with COPA was significantly less than with wild-type SURF4, demonstrating the importance of the dilysine motif to the SURF4-COPA interaction (Fig. 2 E). Consistent with this, we found that the association between SURF4 and mutant COPA was markedly reduced in comparison to wild-type COPA, reflecting a defect in binding between the mutant COPA WD40 domain and SURF4 dilysine tag (Fig. 2 E). Finally, as further evidence that SURF4 functions as an adapter molecule for STING and COPA, loss of SURF4 led to a reduction in the amount of STING that coimmunoprecipitated with wild-type COPA (Fig. S2 C) and also led to an increase in transcript levels of Ifnb1 and type I ISGs (Fig. S2 D). In aggregate, our data suggest that SURF4 functions as a cargo receptor for STING and that mutant COPA is unable to bind SURF4 and incorporate STING into COPI vesicles. This results in retention of STING on the Golgi, where it becomes spontaneously activated and triggers type I interferon signaling.

We next turned to a mouse model of COPA syndrome to understand how mutant COPA-mediated STING activation causes immune dysregulation in vivo. We previously reported that CopaE241K/+ mice, which express one of the same disease-causing mutations as patients, spontaneously develop activated cytokine-secreting T cells and T cell-mediated lung disease (Deng et al., 2020). Through bone marrow chimera and thymic transplant experiments, we showed that mutant COPA within thymic epithelial cells perturbs thymocyte development and leads to a defect in immune tolerance. Because STING is highly expressed in thymic tissue (Manils et al., 2017), we wondered how missorting of STING due to mutant COPA might contribute to the T cell phenotypes we observed in CopaE241K/+ mice.

To evaluate this, we first confirmed that CopaE241K/+ mice demonstrate retention of activated STING on the Golgi with elevated type I interferon signaling, similar to what we observed in patient cells and our transfection assays (Fig. 3, A–C). We next performed bulk RNA sequencing to identify gene expression programs that were altered in thymic epithelial cells. Among the differentially expressed genes, we found significant up-regulation of Ifnb and several type I ISGs, consistent with STING activation (Fig. 4 A). Thymocytes migrating through the thymic epithelium were impacted by higher Ifnb levels because they exhibited an elevated type I interferon signature (Fig. 4 B) and higher levels of Qa2 (Fig. 4 C), a cell surface marker expressed in response to IFN-β. We examined thymocyte populations using a staining protocol that subsets increasingly mature single positive (SP) cells into semi-mature (SM), mature 1 (M1), and mature 2 (M2) populations (Fig. 4 D). Prior studies found that type I interferons are required during the transition of thymocytes from SM to M2 cells (Xing et al., 2016). In CopaE241K/+ mice, we found a significant increase in the proportion of M2 cells (Fig. 4 D), suggesting that higher IFN-β levels promoted an expansion of late-stage thymocytes.
Figure 4. Activated STING perturbs thymocyte development by increasing type I interferons in the thymus. (A) Medullary thymic epithelial cells sorted from WT and CopaE241K/+ mice were used for RNA-sequencing analysis (n = 3 each genotype). Volcano plot of the differentially affected genes (FDR < 0.1, red; FDR < 0.1, ISG from viral response Gene Ontology categories, purple). (B) Real-time PCR performed for Ifit1, Rsad2, and Isg15 expression in SP thymocytes (top: CD4SP; bottom: CD8SP). n = 4 mice each genotype, two independent experiments. (C) Left: Percentages of Qa2high population among SP thymocytes (top: CD4SP; bottom: CD8SP). n = 5 each genotype, three independent experiments. Right: Representative flow analysis of Qa2 expression on SP thymocytes (top: CD4SP; bottom: CD8SP). (D) Top: CD69 versus MHCI expression on SP thymocytes (top: CD4SP; bottom: CD8SP). Bottom: Percentages of SM and M2
To determine the role of STING in these changes, we crossed Copa241K/− mice to STING-deficient Sting1gt/gt mice. We measured mRNA transcripts of thymic epithelial cells for Ifnβ and type I ISGs and found that all of the increases returned to wild-type levels in Copa241K/− × Sting1gt/gt mice (Fig. 3 E). An analysis of peripheral T cell populations revealed that STING deficiency reversed the significant increase in activated effector memory cells and cytokine-secreting T cells caused by mutant COPA (Fig. 3 A and Fig. S3, B–D). Finally, in strong support of STING being a critical mediator of disease in COPA syndrome pathogenesis, we found that loss of STING rescued embryonic lethality of homozygous Copa241K/− mice. We recovered no Copa241K/241K pups in over five litters from our Copa241K/− × Copa241K/− breeding, although 17 Copa241K/241K mice were born out of 40 pups in five litters from Copa241K/− × Sting1gt/gt parents. In aggregate, these data indicate that STING contributes to immune dysregulation in Copa241K/− mice and that targeting STING in COPA syndrome may be an important therapy for patients.

Activation of STING requires palmitoylation at the Golgi. Defects in COPA function cause STING to multimerize and become spontaneously activated at the Golgi even in the absence of STING ligand, suggesting that at steady state, low levels of STING continuously cycle between the ER and Golgi. In support of our findings, Mukai et al. used cultured cells to show that retrograde transport by COP1 is essential for maintaining STING in its dormant state, independent of cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) synthase (Mukai et al., 2020 Preprint). Although we used a candidate approach to identify SURF4 as a putative cargo receptor that engages STING for incorporation into COPI vesicles by COPA, Mukai et al. directly tested 18 STING-binding proteins containing C-terminal dilysine motifs and found that knockdown of SURF4 was the only one that caused STING to localize on the Golgi. Future studies should address in greater depth whether SURF4 is a central mediator in STING transport and if other adapter molecules are involved. Taken together, our data suggest a role for SURF4 in mediating the retrieval of STING from the Golgi by COP1 and provide new insight into the steady-state dynamics of STING transport in resting cells.

Missense mutations in COPA that lie in the WD40 domain impair retrograde transport of a broad range of COPA cargo proteins containing C-terminal dilysine motifs (Watkin et al., 2015). Despite this, our data suggest that impaired trafficking of STING in particular is key to the pathogenesis of COPA syndrome, since not only does loss of STING reverse many of the immunological derangements in our mouse model, but it also strikingly rescues embryonic lethality of homozygous mutant mice. Further study should be undertaken to determine whether other COPA cargo contributes to COPA syndrome pathogenesis independent of STING.

The ubiquitous expression of COPA has made it difficult to identify the cell types that are responsible for causing disease, particularly since COPA is not enriched in any specific immune or lung cell. The tissue specificity of STING may provide additional insight into the understanding of COPA syndrome and the organs most affected. One unexpected outcome of our work was the finding that STING has a functional role in thymic stromal tissue. Although thymic epithelial cells are known to be a significant source of type I interferons (Otero et al., 2013; Xing et al., 2016), the mechanisms regulating interferon secretion in the thymus remain largely unexplored. Interestingly, STING also modulates autophagy (Gui et al., 2019), which in thymic epithelial cells is essential for processing self-antigen peptides for presentation to thymocytes (Nedjic et al., 2008). Additional
Figure 5. **Loss of STING function dampens inflammation caused by mutant COPA.** (A) Top left: Intracellular levels of IFN-γ and IL17A in splenic CD4+ T cells after PMA/ionomycin stimulation. Top right: Percentages of IFN-γ–producing CD4+ T cells. n ≥ 3 each genotype, more than three independent experiments. Bottom left: Intracellular IFN-γ production in splenic CD8+ T cells after PMA/ionomycin stimulation. Bottom right: percentages of IFN-γ–producing CD8+ T cells. n ≥ 3 each genotype, more than three independent experiments. (B) Real-time PCR for ISG expression in splenocytes harvested from four WT mice and four CopaE241K/+ mice treated with or without STING inhibitor C-176 (10 µM) for 24 h. (C and D) Real-time PCR performed in duplicate for ISG expression in PBMCs from a healthy control and a COPA syndrome subject treated with or without STING inhibitor H-151 (10 µM; C) or JAK inhibitor tofacitinib (2 µM; D) for 24 h. Data represent means ± SD. **P < 0.01, ***P < 0.001; ns, not significant (unpaired, parametric, two-tailed Student’s t tests).
research might address whether activated STING alters autophagic function in thymic epithelial cells and, if so, whether this impacts T cell selection.

Our work indicates that COPA syndrome belongs to a category of diseases defined by STING activation (Uggetti et al., 2019). Going forward, clinicians may want to compare and contrast clinical features and treatment approaches in COPA syndrome and SAVI to establish optimal care of patients. Understanding the mechanisms by which STING causes interstitial lung disease has the potential to substantially improve outcomes in both disorders (Tsui et al., 2018; Liu et al., 2014). For those with COPA syndrome, the dramatic reversal of immune dysregulation that we observed in CopaE241K/+ Sting1gt/gt mice provides some hope that small-molecule STING inhibition can be an effective molecularly targeted approach for treating this highly morbid disease.

Materials and methods

Reagents

2′,3′-cGAMP and CP-690550 (tofacitinib) were purchased from InvivoGen. STING inhibitors C-176 and H-151 were synthesized by SYNthesis med chem at InvivoGen. STING inhibitors C-176 and H-151 were synthesized in both disorders (Tsui et al., 2018; Liu et al., 2014). For those with COPA syndrome, the dramatic reversal of immune dysregulation that we observed in CopaE241K/+ Sting1gt/gt mice provides some hope that small-molecule STING inhibition can be an effective molecularly targeted approach for treating this highly morbid disease.

Plasmids

Human STING and SURF4 were subcloned from HEK293T cDNA into pCMV6-AC (Origene) with a FLAG tag at the C terminus or an HA tag at the N terminus, respectively. Plasmids expressing FLAG-tagged wild-type and mutant E241K human COPA were previously generated (Watkin et al., 2015). Retroviral plasmids expressing EGFP-tagged human and mouse STING were kindly provided by Dr. Tomohiko Taguchi (Tohoku University, Sendai, Japan).

Study subjects

Subjects were selected on the basis of COPA syndrome diagnosis, with their written informed consent, and were studied via protocols approved by the Research Ethics Board of Toronto General Hospital and the Institutional Review Boards for the protection of human subjects of Cleveland Clinic or the University of California, San Francisco (UCSF).

Isolation of human lung fibroblasts

Control lung tissue was harvested from anonymous brain-dead donors from the Northern California Transplant Donor Network. Screening criteria for selection of healthy lungs for specimen collection were previously described (Lee et al., 2009). Mutant lung explants were from two COPA syndrome patients receiving lung transplants. Fibroblasts were isolated as described. In brief, tissue was minced with scissors into 5-mm pieces and digested three times with 0.25% trypsin (GE Healthcare Life Sciences) for 10 min at 37°C. The resulting cell and tissue suspension was collected, neutralized with complete media (45% Ham’s F12, 45% DMEM, and 10% FBS), pelleted, plated onto FBS-coated 60-mm dishes, and cultured in 5% CO2 at 37°C. Fibroblasts were ready to passage and use 1 wk following isolation.

Isolation of MEFs

MEFs were isolated as described (Durkin et al., 2013). In brief, day 13.5 embryos were washed with PBS, minced with scissors into 1-2 mm pieces, and digested three times with 0.25% trypsin for 10 min at 37°C. The cell suspension was neutralized with complete media (DMEM and 10% FBS), pelleted, resuspended in complete media, and cultured in 5% CO2 at 37°C.

To create immortal MEFs, Phoenix packaging cells (kindly gifted by Dr. Mark Anderson, UCSF, San Francisco, CA) were transfected with pBABE-neo largeT cDNA plasmid (a gift from Robert A. Weinberg, Whitehead Institute for Biomedical Research, Cambridge, MA; Addgene plasmid #1780; http://n2t.net/addgene:1780; RRID:Addgene_1780; Hahn et al., 2002), and viral supernatant was collected 2 d later. Early-passage MEFs were incubated in viral supernatant for 2 d, and then transformed cells were selected with G418 (Teknova).

siRNA knockdown

Predesigned siRNA oligomers (ON-TARGETplus SMARTPool) for SURF4 and transfection control (siGENOME RISC-Free) were obtained from Dharmacon and resuspended in RNase-free water at 10 μM. siRNAs were transfected in Opti-Mem media (Life Technologies) for 48 h with Lipofectamine RNAiMAX (Life Technologies) according to manufacturer’s protocol.

Immunoblotting and antibodies

Cells were lysed in Cold Spring Harbor NP-40 lysis buffer (150 mM NaCl, 50 mM Tris, pH 8.0, and 1.0% Nonidet-P40) supplemented with protease and phosphatase inhibitors (PMSF, NaF, Na3VO4, and Roche PhosSTOP) and then centrifuged at 12,000 g for 15 min at 4°C to get cellular lysate. Equal amounts of protein were loaded and size separated on an SDS–PAGE gel and wet transferred onto polyvinylidene fluoride membrane. The membrane was blocked in tris-buffered saline and Tween 20 (TBS-T) buffer with 5% milk for 1 h at room temperature, followed by overnight incubation with primary antibodies diluted in TBS-T with 5% BSA. Membrane was washed three times with TBS-T buffer for 10 min, incubated at room temperature with HRP-conjugated IgG secondary antibody (Jackson Immunoresearch), washed three times with TBS-T for 10 min followed once with TBS buffer for 10 min, and then developed with SuperSignal West Femto Chemiluminescent Substrate (Life Technologies).

Rabbit antibodies against TBK1 (D1B4), pTBK1 (Ser172, D52C2), STING (D2P2), p-STING (Ser365, D8F4W), p-STING (Ser366, D7C8S), FLAG (D6W5B), HA (C29F4), and GFP (D5.1) were from Cell Signaling Technology. Rabbit antibody against SURF4 was from Novus. Mouse antibodies against GAPDH and β-Tubulin were from Santa Cruz Biotechnology. Mouse antibody against GM130 was from BD Biosciences.

Coimmunoprecipitation

HEK293 or HEK293T cells transfected with indicated plasmids were collected, washed once with PBS, lysed in NP-40 lysis buffer supplemented with protease and phosphatase inhibitors, and centrifuged at 12,000 g for 15 min at 4°C. The supernatant was mixed with FLAG-M2 beads (Sigma) and incubated...
overnight at 4°C. 10% of lysate was saved as input. The following day, the beads were washed three times with immunoprecipitate (IP) washing buffer (10 mM Tris, pH 7.4, 1 mM EDTA, 150 mM NaCl, and 1% Triton X-100) for 10 min. 2× SDS loading buffer (100 mM Tris, pH 6.8, 4% SDS, 20% glycerol, 10% β-mercaptoethanol, and 0.1% bromophenol blue) was added into the IP complex and boiled at 95°C for 5 min. Samples were analyzed by immunoblot as described above.

Confocal microscopy
Cells were seeded onto glass coverslips and treated as indicated. Cells then were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100, and blocked with 3% BSA. Slides were incubated with primary antibody overnight at 4°C, incubated with fluorescent-conjugated secondary antibody for 1 h at room temperature, and followed with DAPI incubation for 10 min at room temperature. Slides were then mounted with FluorSave Reagent (Millipore) and kept at 4°C in the dark. Images were captured with a Leica TCS SPE microscope, 63× objective, and oil immersion.

Mice strains
Copa2Δ24/Δ24 knock-in mice were generated in our laboratory (Deng et al., 2020). C57BL/6j-Sting+/− (Sting−/−) mice were purchased from The Jackson Laboratory. All mice were maintained in the specific pathogen-free facility at UCSF, and all protocols were approved by UCSF’s Institutional Animal Care and Use Committee.

Flow cytometry and antibodies
Single-cell suspensions of thymocytes and splenocytes were prepared by mechanically disrupting the thymus and spleen. Cells were filtered through 40-µm filters (Genesee Scientific) into 15-ml conical tubes and maintained in RPMI 1640 containing 5% FBS on ice. Splenocytes were further subjected to red blood cell lysis (Biolegend). For evaluation of surface receptors, cells were blocked with 10 µg/ml anti-CD16/32 for 15 min at room temperature and then stained with indicated antibodies in FACS buffer (PBS and 2% FBS) on ice for 40 min.

For intracellular cytokine detection, freshly isolated splenocytes were stained by PMA (Sigma) and ionomycin (Sigma) in the presence of brefeldin A (Biolegend) for 6 h. Cells were collected and stained with Ghost Dye Violet 450 (Tonbo Biosciences) followed by surface staining. Cells were then washed, collected and enzymatically digested three times (DMEM, 2% FBS, 100 µg/ml DNase I, and 100 µg/ml Liberase). The single-cell suspensions from each digestion were pooled into 20 ml of magnetic-activated cell sorting buffer (PBS, 0.5% BSA, and 2 mM EDTA) on ice. Total cells were washed and centrifugated using a three-layer Percoll gradient with specific gravities of 1.115, 1.065, and 1.0. Thymic stromal cells were enriched and collected from the Percoll-light fraction, between the 1.065 and 1.0 layers. Thymic stromal cells were washed, stained, and sorted to isolate MHC-IIhighCD80high medullary thymic epithelial cells by FACS.

RNA isolation and quantitative real-time PCR analysis
RNA was isolated from the EZTA Total RNA kit (Omega Bio-tek) and was reverse transcribed to cDNA with SuperScript III reverse transcription and oligo d(T)20 primers (Invitrogen). Quantitative real-time PCR was performed on Bio-Rad CFX thermal cyclers with TaqMan Gene Expression assays from Life Technologies (Hs00278662_g1); IFNB1, Hs01077958_s1; IFI6, Hs00242571_ml; IFIT7, Hs01086373_g1; IFIT4, Hs00915292_ml; ICSi5, Hs01921425_s1; ITIT1, Hs00307269_s1; IFTT1, Hs00705137_s1; RSAD2, Hs00369813_ml; Gapdh, Mm99999915_g1; Ifi44, Mm00439552_s1; Ifit3, Mm00515153_ml; Ifi44l, Mm00518988_ml; Isgl5, Mm01705338_s1; Rsad2, Mm00491265_ml; and Slprl, Mm00514644).

RNA sequencing and data analysis
RNA-sequencing libraries were generated by first using the Nugen Ovation method (Tecnion 7102-A01) to create cDNA from the isolated RNA, the sequencing library was then created from cDNA using the Nextera XT method (Illumina; FC-131-1096). All libraries were combined and sequenced on Illumina HiSeq4000 lanes, yielding ~300 million single-end, 50-bp reads. Sequencing reads were then aligned to the mouse reference genome and the ensemble annotation build (GRCm38.78) using STAR (Dobin et al., 2013; v2.4.2a). Read counts per gene were used as input to DESeq2 (Love et al., 2014; v1.26.0) to test for differential gene expression between conditions using a Wald test. Genes passing a multiple-testing correct P value of 0.1 (false discovery rate [FDR] method) were considered significant. Pathway analysis was performed using DAVID (Huang et al., 2007) and the R/Bioconductor package RDAVIDWebService (Fresno and Fernández, 2013; v1.24.0).

Statistical analysis
All statistical analysis was performed using Prism 7 (GraphPad Software) or R 4.0.0 (R Foundation for Statistical Computing). Where indicated, two-tailed Mann-Whitney U test and unpaired, parametric, two-tailed Student’s t test were used to evaluate the statistical significance between two groups, and P < 0.05 was considered statistically significant.

Data availability
The bulk RNA sequencing data are available at the Gene Expression Omnibus (GEO accession no. GSE153822).

Online supplemental material
Fig. S1 shows STING activation in lung fibroblasts isolated from a COPA syndrome patient. Fig. S2 is evidence of SURF4’s role in
STING retrieval via COPA. Fig. S3 demonstrates type I interferon–driven inflammation in Copα2ΔIRK-1/1 mice due to STING activation.

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Author contributions: Z. Deng and Z. Chong designed and performed experiments and analyzed data. K. Mukai and T. Taguchi provided technical advice. C.S. Law performed experimental design. W.L. Eckalbar analyzed RNA-sequencing data. T. Martinu provided lung explant tissue. A.K. Shum directed the study and wrote the manuscript.

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Supplemental material

Figure S1. COPA syndrome patient fibroblasts demonstrate STING activation. (A) Real-time PCR was performed for IFNB1 expression in primary lung fibroblasts from a healthy control and a COPA syndrome subject treated with cGAMP (2 µg/ml) for indicated time. Data from six samples in two independent experiments are means ± SD, two-tailed Mann-Whitney U test. (B) Real-time PCR in triplicate for ISG expression in primary lung fibroblasts from a healthy control and a COPA syndrome subject treated with cGAMP (2 µg/ml) for indicated time. Data are means ± SD, unpaired two-tailed Student’s t test. (C) Immunoblots of primary lung fibroblasts for indicated antibodies. **P < 0.01, ***P < 0.001, ****P < 0.0001.
Figure S2. **SURF4 functions as a cargo receptor that mediates retrieval of STING by COPA.** (A) Seven STING interacting partners with C-terminal dilysine motifs were identified in published studies describing STING affinity purification–mass spectrometry data (Lee et al., 2013; Huttlin et al., 2017; Shang et al., 2018; Li et al., 2011; UniProt Consortium, 2019). Shown for each protein are its C terminus sequence with dilysine motif highlighted, subcellular location, and function. (B) FLAG IPs from lysates of HEK293T cells overexpressing FLAG-tagged STING and HA-tagged SURF4 were immunoblotted for indicated antibodies. (C) HEK293 cells were transfected with FLAG-tagged COPA and si-SURF4 for 48 h. FLAG IPs from lysates of HEK293 cells were immunoblotted for indicated antibodies. (D) Real-time PCR in triplicate for ISG expression in immortalized MEF cells with EGFP-STING after si-SURF4 for 48 h. Data represent means ± SD. *P < 0.05, **P < 0.01 (unpaired two-tailed Student’s t tests). ERGIC, ER-Golgi intermediate compartment; siNeg, nontargeting siRNA control.
Figure S3. Activated STING in \textit{Copa}^{E241K/+} mice results in type I interferon–driven inflammation. (A) Real-time PCR performed for ISG expression in splenocytes from WT (\(n=5\)), \textit{Copa}^{E241K/+} (\(n=5\)), \textit{Sting}^{gt/gt} (\(n=5\)), and \textit{Copa}^{E241K/+}×\textit{Sting}^{gt/gt} (\(n=6\)) mice. (B) Left: Representative flow plots showing expression of CD62L versus CD44 on T cells (top: CD4\(^+\) T cells; bottom: CD8\(^+\) T cells). Right: Percentages of naive and effector memory T cells (left: CD4\(^+\) T cells; right: CD8\(^+\) T cells). WT: \(n=6\); \textit{Copa}^{E241K/+}: \(n=4\); \textit{Sting}^{gt/gt}: \(n=5\); \textit{Copa}^{E241K/+}×\textit{Sting}^{gt/gt}: \(n=5\). (C) Left: Intracellular levels of IL13 in splenic CD4\(^+\) T cells after PMA/ionomycin stimulation. Right: percentages of IL13-producing CD4\(^+\) T cells. WT: \(n=6\); \textit{Copa}^{E241K/+}: \(n=4\); \textit{Sting}^{gt/gt}: \(n=5\); \textit{Copa}^{E241K/+}×\textit{Sting}^{gt/gt}: \(n=5\), more than three independent experiments. (D) Left: Intracellular TNF-\(\alpha\) production in splenic CD8\(^+\) T cells after PMA/ionomycin stimulation. Right: percentages of TNF-\(\alpha\)-producing CD8\(^+\) T cells. WT: \(n=4\); \textit{Copa}^{E241K/+}: \(n=3\); \textit{Sting}^{gt/gt}: \(n=5\); \textit{Copa}^{E241K/+}×\textit{Sting}^{gt/gt}: \(n=5\), more than three independent experiments. Data in A–D represent means ± SD. *\(P < 0.05\), **\(P < 0.01\), ***\(P < 0.001\), ****\(P < 0.0001\); ns, not significant (unpaired, parameteric two-tailed Student’s \(t\) tests).