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IMMUNOLOGY

# cAMP metabolism controls caspase-11 inflammasome activation and pyroptosis in sepsis

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**The ability of cytosolic lipopolysaccharide (LPS) to activate caspase-11–dependent nonclassical inflammasome is intricately controlled to avoid excessive inflammatory responses. However, very little is known about the regulatory role of various metabolic pathways in the control of caspase-11 activation. Here, we demonstrate that L-adrenaline can act on receptor ADRA2B to inhibit the activation of the caspase-11 inflammasome by cytosolic LPS or *Escherichia coli* infection in macrophages. L-adrenaline–induced cAMP production via the enzyme ADCY4 promotes protein kinase A (PKA) activation, which then blocks the caspase-11–mediated proteolytic maturation of interleukin-1β, gasdermin D (GSDMD) cleavage, and consequent DAMP release. Inhibition of PDE8A-mediated cAMP hydrolysis limits caspase-11 inflammasome activation and pyroptosis in macrophages. Consequently, pharmacological modulation of the ADRA2B-ADCY4-PDE8A-PKA axis, knockout of *caspase-11* (*Casp11*$^{-/-}$), or *Gsdmd* inactivation (*Gsdmd*$^{I105N/I105N}$) similarly protects against LPS-induced lethality in poly(I:C)-primed mice. Our results provide previously unidentified mechanistic insight into immune regulation by cAMP and represent a proof of concept that immunometabolism constitutes a potential therapeutic target in sepsis.**

## INTRODUCTION

Macrophages are innate immune cells that play a key role in initiation, amplification, and resolution of inflammatory processes. As an inflammatory cell death pathway, pyroptosis occurs mainly in macrophages and their precursors, monocytes following activation of inflammatory caspase-1 and caspase-11 (caspase-4 and caspase-5 in humans), and cleavage-mediated activation of GSDMD (gasdermin D) (*1*, *2*). The resultant N-terminal GSDMD fragment, GSDMD-N, oligomerizes to form pores in the plasma membrane, resulting in its permeabilization and lytic cell death (*1*–*4*). Thus, inflammasomes not only mediate proteolytic processing and secretion of IL-1 (interleukin-1) family cytokines (e.g., IL-1β and IL-18) but also cause the cell death–associated release of damage-associated molecular patterns [e.g., LDH (lactate dehydrogenase) and HMGB1 (high-mobility group box 1)]. Excessive activation of inflammasomes and consequent pyroptosis are implicated in human diseases and conditions, including sepsis (*5*).

Sepsis from microbial infection remains a prominent cause of death of critically ill patients in the intensive care unit. Lipopolysaccharide (LPS) is a component of the outer membrane of Gram-negative bacteria, one of the leading pathogens responsible for sepsis. LPS initiates immediate innate immune responses not only through cell surface TLR4 (Toll-like receptor 4) to induce inflammation-related gene expression (*6*) but also via the activation of a cytosolic receptor, caspase-11, to trigger nonclassical inflammasome activation (*7*–*11*). Cytosolic LPS can cause caspase-11 oligomerization and inflammasome activation, leading to the generation of GSDMD-N and subsequent pyroptosis in macrophages (*11*). Mice genetically deficient in caspase-11, but not caspase-1, are resistant to LPS-induced septic shock (*7*, *10*, *12*). This cytosolic LPS–caspase-11–sensing pathway represents not only a important paradigm in innate immunity but also a potential target for therapeutic exploration in sepsis. However, the metabolic regulation of the caspase-11 inflammasome has not been elucidated.

Cyclic adenosine monophosphate (cAMP) is one of the principal second messengers generated in response to hormones and acts intracellularly by activating its effectors PKA (protein kinase A) and RAPGEF3/EPAC (rap guanine nucleotide exchange factor 3). cAMP is synthesized from adenosine triphosphate by enzymes from the ADCY (adenylyl cyclase) family and can be hydrolyzed by a series of enzymes from the phosphodiesterase (PDE) family (*13*). In this study, we provide evidence that the surge in intracellular cAMP stimulated by the stress hormone and neurotransmitter L-adrenaline–induced cAMP can control cytosolic LPS-induced activation of the caspase-11 inflammasome and consequent pyroptosis in macrophages. Targeting the ADCY4-PDE8A-PKA axis protects mice from caspase-11–dependent septic death. Our data therefore shed previously unidentified mechanistic insight into the metabolic modulation of the caspase-11 inflammasome and provide a potential therapeutic strategy for the treatment of lethal infection.

## RESULTS

### Identification of L-adrenaline as an inhibitor of the caspase-11 inflammasome

Activation of the caspase-11–dependent noncanonical inflammasome requires two signals: a priming signal (e.g., TLR ligands) that induces

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the transcriptional up-regulation of inflammasome components and then a sensing signal (e.g., LPS electroporation or *Escherichia coli* infection) that triggers caspase-11 activation and pyroptosis (*7*–*10*, *12*). To identify potential regulators of caspase-11 inflammasome in innate immune cells, we screened a U.S. Food and Drug Administration (FDA)–approved library of 1018 drugs in murine bone marrow–derived macrophages (BMDMs) that were first primed by exposure to extracellular LPS and then electroporated with LPS ($LPS_e$). The $LPS_e$-induced cell growth inhibition was reversed by certain drugs (Fig. 1A). The top five drugs that counteracted $LPS_e$-induced growth inhibition in LPS-primed BMDMs included L-adrenaline (a stress hormone, also termed epinephrine), levosulpiride (an antipsychotic agent), tetracaine (a local anesthetic), prednisolone (a synthetic glucocorticoid), and quinapril (an angiotensin-converting enzyme inhibitor) (Fig. 1B). These drugs also prevented $LPS_e$-induced cell death in the human monocytic cell line, THP1 (Fig. 1B). LDH and IL-1β release assay indicated that $LPS_e$-induced cell death and cytokine release in BMDMs and THP1 cells were blocked by these top five drugs (Fig. 1B). L-adrenaline was also active when it was tested on BMDMs that were primed with Pam3CSK4 (a TLR1 and TLR2 ligand) or poly(I:C) (a TLR3 ligand) instead of LPS. Irrespective of the priming signal, L-adrenaline inhibited the cytotoxicity and IL-1β release of $LPS_e$ or *E. coli* infection (Fig. 1C). Similar to BMDMs and THP1 cells, L-adrenaline also inhibited $LPS_e$-induced cytotoxicity and IL-1β release in human primary monocytes (fig. S1). Immunoblot analysis showed that L-adrenaline also inhibited the $LPS_e$- or *E. coli*–induced caspase-11 activation (yielding a p26 band), GSDMD-N formation, and proteolytic IL-1β maturation (yielding a p17 fragment) in LPS-primed BMDMs (Fig. 1D). As expected, caspase-11 and GSDMD, but not canonical inflammasome proteins [e.g., NLRP3 (NLR family pyrin domain containing 3), NLRC4 (NLR family CARD domain containing 4), and NLRP1], were required for the killing of LPS-primed BMDMs by $LPS_e$ or *E. coli* (Fig. 1E). L-adrenaline failed to affect LPS-induced pro–caspase-11 and pro–IL-1β expression during the priming stage of inflammasome activation (fig. S2). Collectively, these findings suggest that L-adrenaline acts as an inhibitor of caspase-11 inflammasome activation in macrophages and monocytes.

## Adrenoceptor α 2B is required for L-adrenaline–mediated blockade of caspase-11 inflammasome activation

The adrenergic receptors are a class of G protein (heterotrimeric GTP-binding protein)–coupled receptors for endogenous catecholamines, including L-adrenaline. To identify the adrenergic receptor responsible for L-adrenaline–mediated inhibition of caspase-11 inflammasome activation, we first analyzed adrenergic receptor expression in activated innate immune cells. Quantitative real-time polymerase chain reaction (qPCR) analysis showed that the mRNA expression of adrenoceptor α 2B (*Adra2B*), but not that of other adrenergic receptors (*Adrb1*, *Adrb2*, *Adrb3*, *Adra2a*, *Adra2c*, *Adra1a*, *Adra1b*, or *Adra1d*), was up-regulated in BMDMs in response to $LPS_e$ combined with L-adrenaline (Fig. 2A). Knockdown of *Adra2b* (but not *Adra2a*) by small interfering RNA (siRNA) pools (Fig. 2B) reversed the L-adrenaline–mediated inhibition of LDH and IL-1β release from BMDMS responding to $LPS_e$ or *E. coli* (Fig. 2C). Western blot analysis confirmed that *Adra2b* knockdown abrogated the inhibitory effect of L-adrenaline on $LPS_e$-induced caspase-11 (p26) activation, GSDMD-N formation, and proteolytic IL-1β maturation (p17) (Fig. 2D). These findings suggest that ADRA2B mediates L-adrenaline activity to counter-regulate the cytoplasmic LPS-mediated caspase-11 activation and pyroptosis.

## ADCY4-mediated cAMP synthesis inhibits caspase-11 inflammasome activation

L-adrenaline acts as the "flight or fight hormone" in response to stress and promotes rapid, close-to-immediate rises in intracellular cAMP, a process that acts as the major molecular mechanism of signal transduction. As predicted, L-adrenaline increased cAMP levels in BMDMs responding to $LPS_e$ or *E. coli* (Fig. 3A). Knockdown of *Adra2b* (but not *Adra2a*) suppressed this L-adrenaline–elicited cAMP elevation, confirming that ADRA2B mediates the L-adrenaline effects on this system (Fig. 3A). To evaluate the regulatory role of cAMP on $LPS_e$-induced caspase-11 activation, we used cell-permeable cAMP analogs. Several cAMP analogs such as 8-bromoadenosine–3′,5′-cyclic monophosphate sodium salt (8-Br-cAMP) and $N^6$-benzoyladenosine–3′,5′-cyclic monophosphate sodium salt (6-Bn-cAMP) similarly inhibited $LPS_e$- or *E. coli*–induced LDH and IL-1β release from BMDMs and THP1 cells (Fig. 3B). The $LPS_e$-induced caspase-11 (p26) activation, GSDMD-N formation, and proteolytic IL-1β maturation (p17) were also inhibited by 8-Br-cAMP or 6-Bn-cAMP (Fig. 3C). These findings suggest that cAMP can act as an endogenous inhibitor of caspase-11–mediated inflammasome activation.

The synthesis of cAMP elicited by hormone-receptor interactions requires the enzymatic activity of proteins from the ADCY. There are 10 ADCY family members in mammalian cells. qPCR analysis showed that the mRNA expression of *Adcy4*, but not any other ADCY members, was up-regulated in BMDMs responding to $LPS_e$ combined with L-adrenaline (Fig. 3D). Notably, knockdown of *Adcy4* (but not *Adcy2*) expression (Fig. 3E) blocked cAMP production and restored the LDH and IL-1β release in response to $LPS_e$ or *E. coli* combined with L-adrenaline (Fig. 3F). Immunoblot revealed that $LPS_e$-induced and caspase-11 (p26) activation, GSDMD-N formation, and proteolytic IL-1β maturation (p17) were not inhibited any more by L-adrenaline in LPS-primed BMDMs when *Adcy4* was depleted (Fig. 3G). It thus appears that ADCY4 is required for L-adrenaline–induced cAMP synthesis to limit cytosolic LPS-induced caspase-11 inflammasome activation and pyroptosis.

## PDE8A-mediated cAMP hydrolysis promotes activation of the caspase-11 inflammasome

To further define the role of cAMP metabolism in caspase-11 inflammasome activation, we investigated the impact of cAMP hydrolysis on cytosolic LPS-induced pyroptosis. PDEs are the only enzymes known to degrade cAMP and cGMP (guanosine 3′,5′-monophosphate) in mammalian cells (*13*). One hundred members of the PDE superfamily are divided into 11 subfamilies. Among them, PDE4 (PDE4A-D), PDE7 (PDE7A and PDE7B), and PDE8 (PDE8A and PDE8B) are cAMP-selective hydrolases. $LPS_e$ increased *Pde8a* mRNA expression, whereas L-adrenaline inhibited this process (Fig. 4A). In contrast, other mRNAs of cAMP-selective hydrolases—including *Pde4a*, *Pde4b*, *Pde4c*, *Pde4d*, *Pde7a*, *Pde7b*, and *Pde8b*—were not notably altered by $LPS_e$ combined with L-adrenaline (Fig. 4A), pointing to a specific role of PDE8A in the control of cAMP hydrolysis.

To determine the potential relationship between PDE8A and cAMP in the regulation of caspase-11–mediated inflammasome activation, we first added PF-04957325, a PDE8-selective inhibitor, to BMDMs and THP1 cells. Administration of PF-04957325 increased cAMP levels and inhibited LDH and IL-1β release in response to $LPS_e$ or

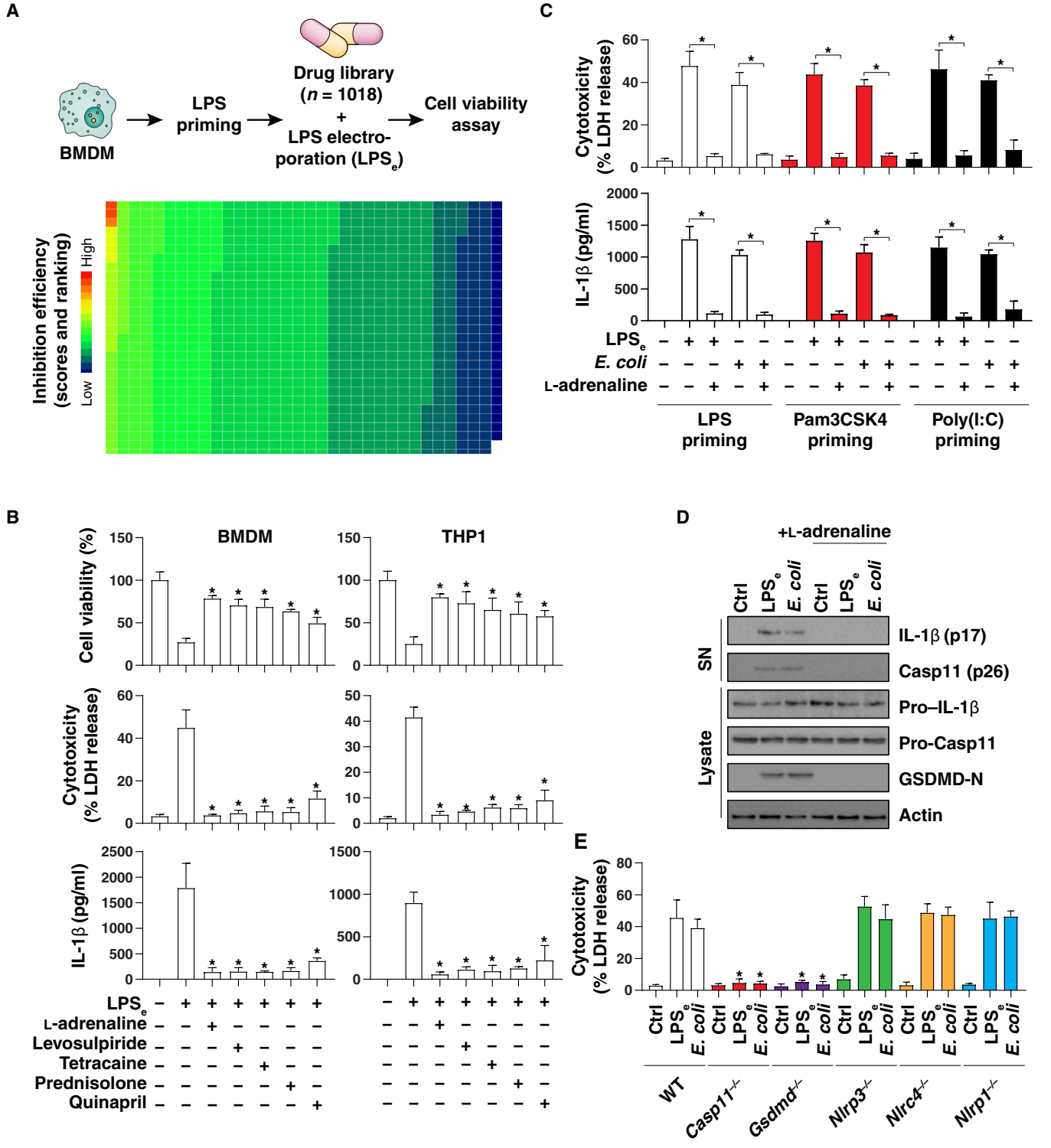


**Fig. 1. Identification of L-adrenaline as a caspase-11 inflammasome inhibitor.** (**A**) Heatmap of cell viability changes in LPS-primed BMDMs after LPS electroporation (1 μg, 16 hours) in the absence or presence of 1018 FDA-approved drugs (10 μM). (**B**) Analysis of cell viability, LDH release, and IL-1β release in LPS-primed BMDMs or THP1 cells after LPS electroporation (1 μg, 16 hours) in the absence or presence of indicated drugs (10 μM). $n = 3$, data expressed as means ± SD; $^*P < 0.05$ versus LPS electroporation group, *t* test. (**C**) Analysis of LDH and IL-1β release in LPS-, poly(I:C)-, or Pam3CSK4-primed BMDMs after LPS electroporation (1 μg, 16 hours) or *E. coli* [multiplicity of infection (MOI), 25; 16 hours] infection in the absence or presence of L-adrenaline (10 μM). $n = 3$, data expressed as means ± SD; $^*P < 0.05$, *t* test. (**D**) Western blot analysis of indicated proteins in the supernatant (SN) or cell lysate in LPS-primed BMDMs after LPS electroporation (1 μg, 16 hours) or *E. coli* (MOI, 25; 16 hours) infection in the absence or presence of L-adrenaline (10 μM). (**E**) Analysis of LDH release in indicated LPS-primed BMDMs after LPS electroporation (1 μg, 16 hours) or *E. coli* (MOI, 25; 16 hours) infection. $n = 3$, data expressed as means ± SD; $^*P < 0.05$ versus wild-type (WT) group, *t* test. Ctrl, control.

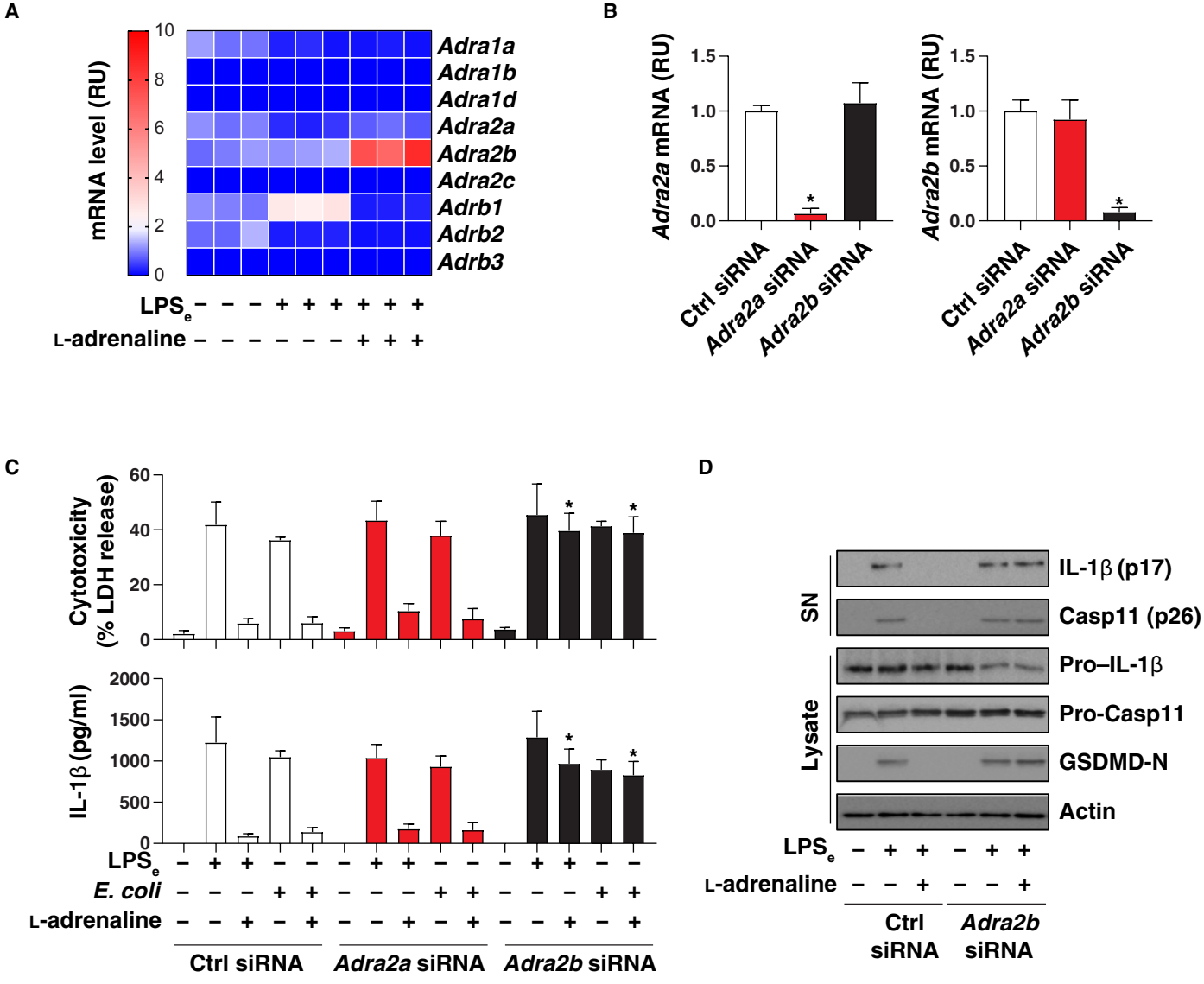


**Fig. 2. ADRA2B is required for L-adrenaline activity in blocking caspase-11 inflammasome.** (**A**) Heatmap of mRNA changes of L-adrenaline receptor in LPS-primed BMDMs after LPS electroporation (1 μg, 16 hours) in the absence or presence of L-adrenaline (10 μM). RU, relative units. (**B**) qPCR analysis of *Adra2a* or *Adra2b* mRNA expression in indicated BMDMs. $n = 3$, data expressed as means ± SD; $^*P < 0.05$ versus control siRNA group, *t* test. (**C**) Analysis of LDH and IL-1β release in indicated LPS-primed BMDMs after LPS electroporation (1 μg, 16 hours) or *E. coli* (MOI, 25; 16 hours) infection in the absence or presence of L-adrenaline (10 μM). $n = 3$, data expressed as means ± SD; $^*P < 0.05$ versus control siRNA group, *t* test. (**D**) Western blot analysis of indicated proteins in the supernatant or cell lysate in indicated LPS-primed BMDMs after LPS electroporation (1 μg, 16 hours) in the absence or presence of L-adrenaline (10 μM).

*E. coli* (Fig. 4B). Knockdown of *Pde8a*, but not *Pde8b*, (Fig. 4C) also increased the intracellular cAMP levels and inhibited LDH and IL-1β release in response to $LPS_e$ or *E. coli* (Fig. 4D). These findings, combined with Western blot analysis to assess the activation of caspase-11 (p26) and the generation of its products GSDMD-N and proteolytically mature IL-1β (p17) (Fig. 4E), indicate that PDE8A is a positive regulator of cytosolic LPS-induced pyroptosis.

### PKA negatively regulates caspase-11 inflammasome activation

To further explore the mechanism of cAMP control of caspase-11 inflammasome activation, we measured the activity of PKA, which is known as a classical cAMP-responsive enzyme (*14*). PKA activity was down-regulated in BMDMs and THP1 cells in response to $LPS_e$ or *E. coli* infection. In contrast, PKA activity was restored upon treatment with L-adrenaline, 8-Br-cAMP, 6-Bn-cAMP, or PF-04957325 (Fig. 5A). In addition, knockdown of *Adra2b* or *Adcy4* reversed the positive effect of L-adrenaline on PKA activity in this system (Fig. 5B), supporting the idea that ADRA2B-ADCY4-PDE8A–dependent cAMP metabolism pathway controls the PKA activity.

Upon binding of cAMP to PKA regulatory subunits, the catalytic subunits are released as active monomers (*14*). To evaluate the role of PKA in the regulation of cytosolic LPS-induced inflammasome activation and pyroptosis, we pharmacologically inhibited PKA with

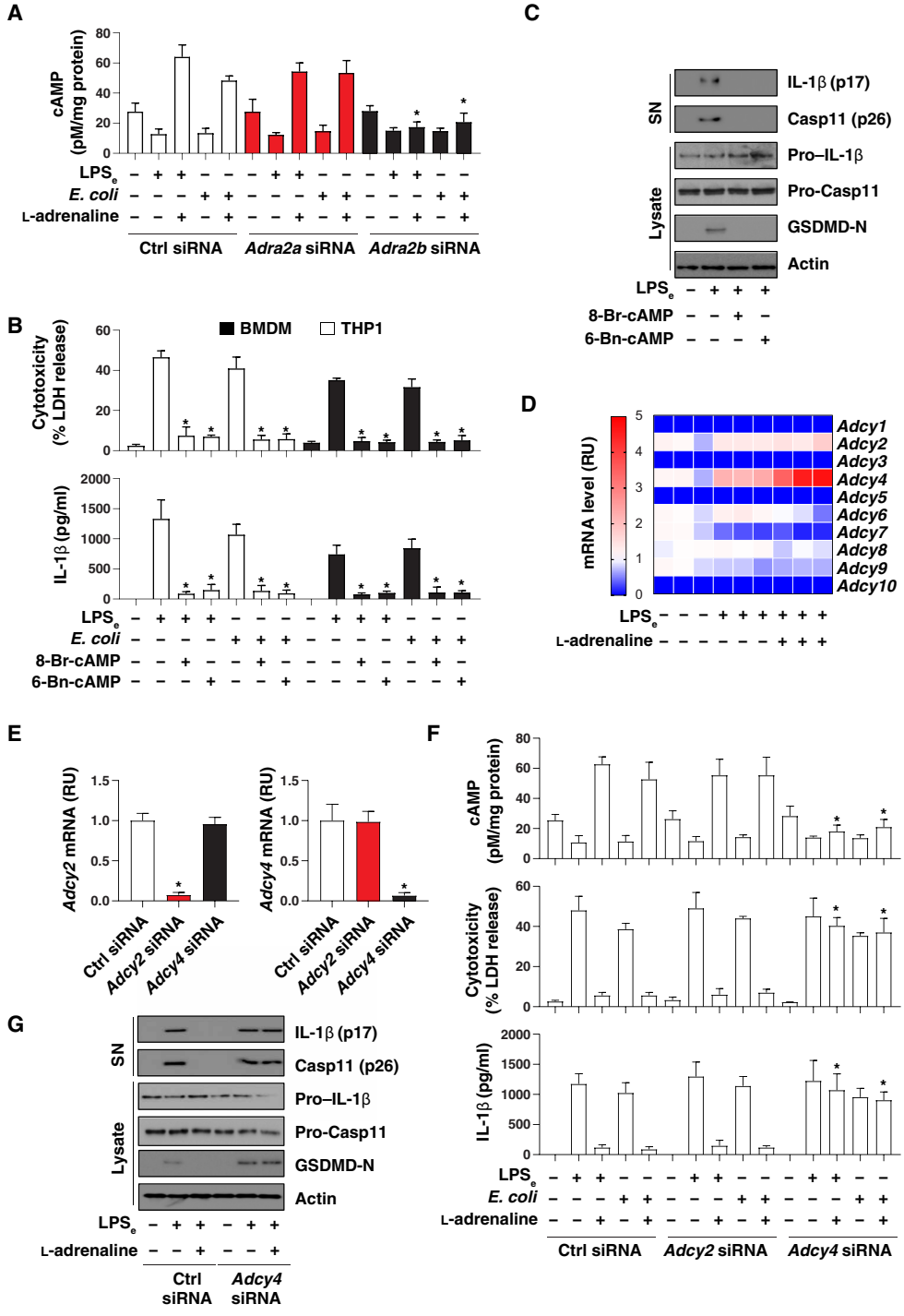


**Fig. 3. ADCY4-mediated cAMP synthesis inhibits caspase-11 inflammasome activation.** (**A**) Analysis of cAMP levels in indicated LPS-primed BMDMs after LPS electroporation (1 μg, 16 hours) or *E. coli* (MOI, 25; 16 hours) infection in the absence or presence of L-adrenaline (10 μM). $n = 3$, data expressed as means ± SD; $^*P < 0.05$ versus control siRNA group, *t* test. (**B**) Analysis of LDH and IL-1β release in indicated LPS-primed BMDMs or THP1 cells after LPS electroporation (1 μg, 16 hours) or *E. coli* (MOI, 25; 16 hours) infection in the absence or presence of indicated cAMP analog (1 mM). $n = 3$, data expressed as means ± SD; $^*P < 0.05$ versus $LPS_e$ or *E. coli* group, *t* test. (**C**) Western blot analysis of indicated proteins in the supernatant or cell lysate in LPS-primed BMDMs after LPS electroporation (1 μg, 16 hours) in the absence or presence of indicated cAMP analog (1 mM). (**D**) Heatmap of mRNA changes of ADCY family in LPS-primed BMDMs after LPS electroporation (1 μg, 16 hours) in the absence or presence of L-adrenaline (10 μM). (**E**) qPCR analysis of *Adcy2* or *Adcy4* mRNA expression in indicated BMDMs. $n = 3$, data expressed as means ± SD; $^*P < 0.05$ versus control siRNA group, *t* test. (**F**) Analysis of cAMP level and LDH and IL-1β release in indicated LPS-primed BMDMs after LPS electroporation (1 μg, 16 hours) or *E. coli* (MOI, 25; 16 hours) infection in the absence or presence of L-adrenaline (10 μM). $n = 3$, data expressed as means ± SD; $^*P < 0.05$ versus control siRNA group, *t* test. (**G**) Western blot analysis of indicated proteins in the supernatant or cell lysate in indicated LPS-primed BMDMs after LPS electroporation (1 μg, 16 hours) in the absence or presence of L-adrenaline (10 μM).

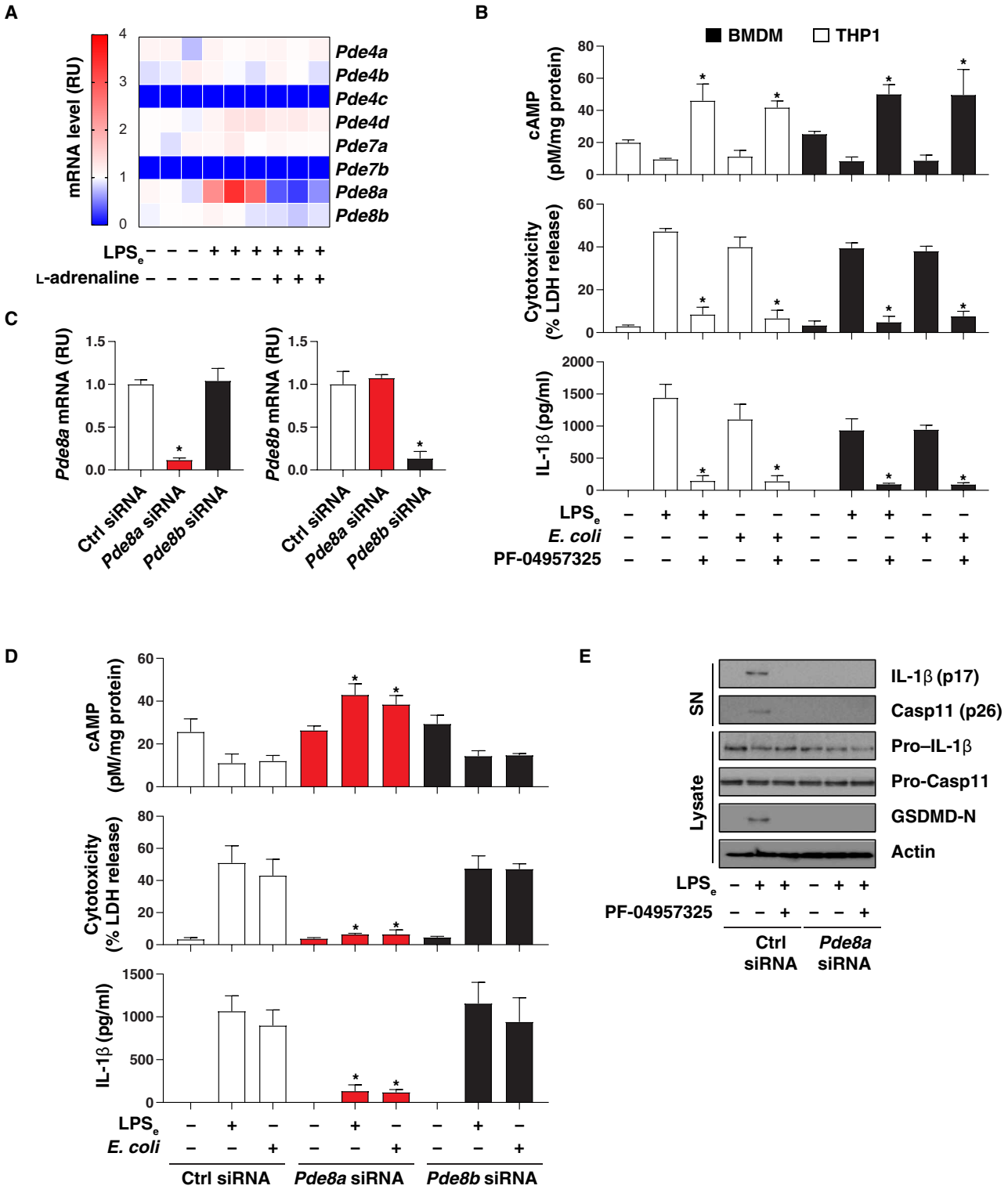


**Fig. 4. PDE8A-mediated cAMP hydrolysis promotes caspase-11 inflammasome activation.** (**A**) Heatmap of PDE family mRNA changes in LPS-primed BMDMs after LPS electroporation (1 μg, 16 hours) in the absence or presence of L-adrenaline (10 μM). (**B**) Analysis of cAMP level and LDH and IL-1β release in LPS-primed BMDMs or THP1 cells after LPS electroporation (1 μg, 16 hours) or *E. coli* (MOI, 25; 16 hours) infection in the absence or presence of PF-04957325 (100 nM). $n = 3$, data expressed as means ± SD; *$P < 0.05$ versus $LPS_e$ or *E. coli* group, *t* test. (**C**) qPCR analysis of *Pde8a* and *Pde8b* mRNA expression in indicated BMDMs. $n = 3$, data expressed as means ± SD; *$P < 0.05$ versus control siRNA group, *t* test. (**D**) Analysis of cAMP level and LDH and IL-1β release in indicated LPS-primed BMDMs after LPS electroporation (1 μg, 16 hours) or *E. coli* (MOI, 25; 16 hours) infection. $n = 3$, data expressed as means ± SD; *$P < 0.05$ versus control siRNA group, *t* test. (**E**) Western blot analysis of indicated proteins in the supernatant or cell lysate in indicated LPS-primed BMDMs after LPS electroporation (1 μg, 16 hours) in the absence or presence of PF-04957325 (100 nM).

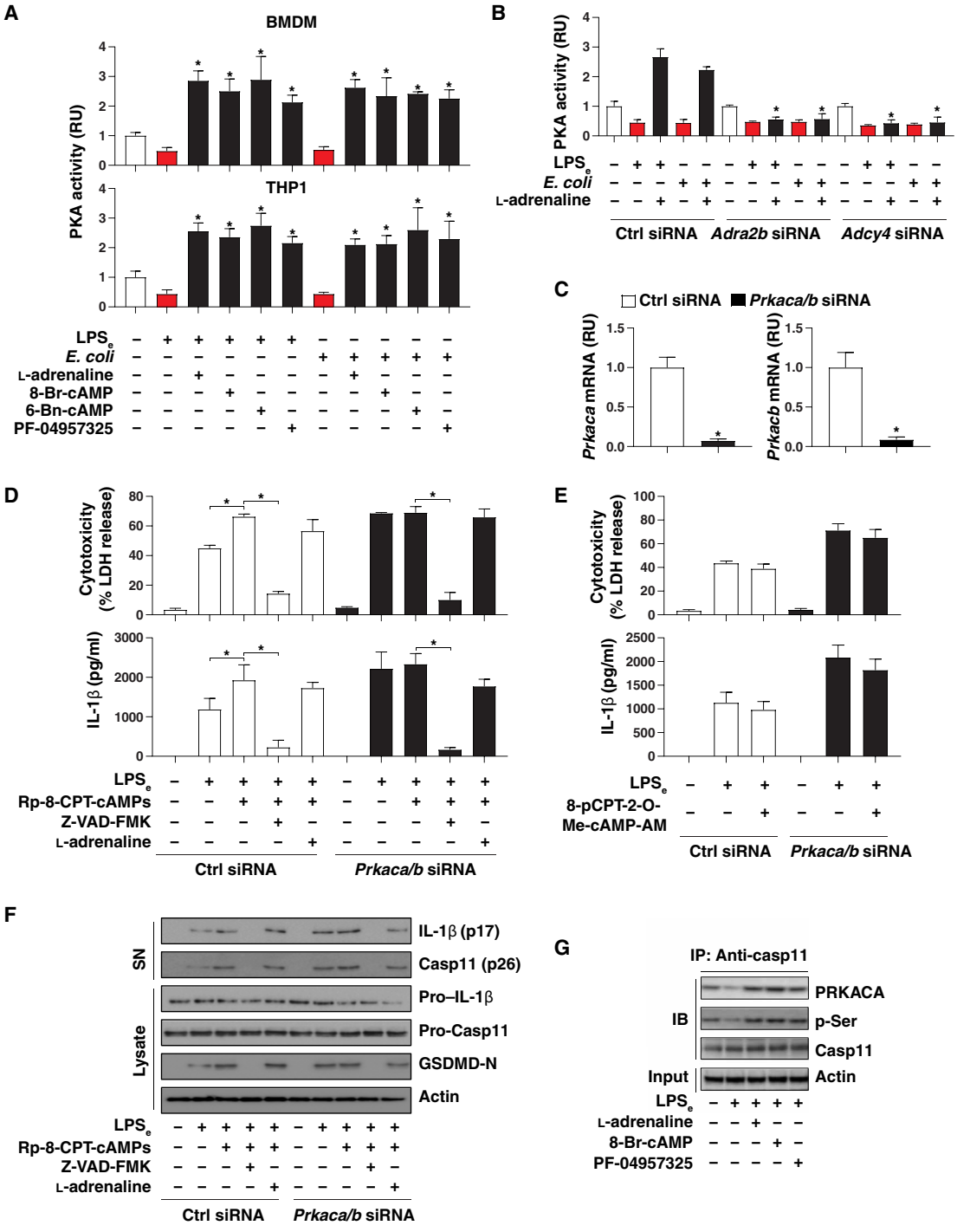


**Fig. 5. PKA negatively regulates caspase-11 inflammasome activation.** (**A**) Analysis of PKA activity in LPS-primed BMDMs or THP1 cells after LPS electroporation (1 μg, 16 hours) or *E. coli* (MOI, 25; 16 hours) infection in the absence or presence of L-adrenaline (10 μM), 8-Br-cAMP (1 mM), 6-Bn-cAMP (1 mM), or PF-04957325 (100 nM). $n = 3$, data expressed as means ± SD; $^*P < 0.05$ versus $LPS_e$ or *E. coli* group, *t* test. (**B**) Analysis of PKA activity in indicated LPS-primed BMDMs after LPS electroporation (1 μg, 16 hours) or *E. coli* (MOI, 25; 16 hours) infection in the absence or presence of L-adrenaline (10 μM). $n = 3$, data expressed as means ± SD; $^*P < 0.05$ versus control siRNA group, *t* test. (**C**) qPCR analysis of *Prkaca* and *Prkacb* mRNA expression in indicated BMDMs. $n = 3$, data expressed as means ± SD; $^*P < 0.05$ versus control siRNA group, *t* test. (**D**) Analysis of LDH and IL-1β release in indicated LPS-primed BMDMs after LPS electroporation (1 μg, 16 hours) in the absence or presence of Rp-8-CPT-cAMPs (300 μM), Z-VAD-FMK (50 μM), or L-adrenaline (10 μM). $n = 3$, data expressed as means ± SD; $^*P < 0.05$, *t* test. (**E**) Analysis of LDH and IL-1β release in indicated LPS-primed BMDMs after LPS electroporation (1 μg, 16 hours) in the absence or presence of 8-pCPT-2-O-Me-cAMP-AM (10 μM). $n = 3$, data expressed as means ± SD. (**F**) Western blot analysis of indicated proteins in the supernatant or cell lysate in indicated LPS-primed BMDMs after LPS electroporation (1 μg, 16 hours) in the absence or presence of Rp-8-CPT-cAMPs (300 μM), Z-VAD-FMK (50 μM), or L-adrenaline (10 μM). (**G**) Immunoprecipitation (IP) analysis of PRKACA–caspase-11 complex and p-Ser (p–caspase-11) in LPS-primed BMDMs after LPS electroporation (1 μg, 16 hours) in the absence or presence of L-adrenaline (10 μM), 8-Br-cAMP (1 mM), or PF-04957325 (100 nM). IB, immunoblot.

Rp-8-CPT-cAMPs or depleted its catalytic subunit α (*Prkaca*) and β (*Prkacb*) with suitable siRNAs (Fig. 5C). Inhibition of PKA increased the $LPS_e$-induced LDH and IL-1β release; this process was reversed by pan-caspase inhibitor Z-VAD-FMK but not by L-adrenaline (Fig. 5D). In contrast, the selective RAPGEF3 activator 8-pCPT-2-O-Me-cAMP-AM had no effect on $LPS_e$-induced LDH and IL-1β release with or without knockdown of *Prkaca/b* (Fig. 5E). Moreover, $LPS_e$-induced caspase-11 (p26) activation, GSDMD-N formation, and proteolytic IL-1β maturation (p17) were increased after inhibition of PKA by Rp-8-CPT-cAMPs or knockdown of *Prkaca/b* (Fig. 5F). Immunoprecipitation analysis further found that the activation of the cAMP pathway by L-adrenaline, 8-Br-cAMP, or PF-04957325 restored the PRKACA–capase-11 complex formation and caspase-11 phosphorylation (p-Ser) in response to $LPS_e$ (Fig. 5G). These observations indicate that the phosphorylation and inhibition of caspase-11 by PKA restrain cytosolic LPS-induced pyroptosis.

### Targeting the cAMP metabolism pathway attenuates caspase-11–mediated lethal endotoxic shock

We next investigated the relevance of our in vitro findings in a mouse model of acute endotoxic shock. We primed mice by intraperitoneal injection of poly(I:C) and subsequently challenged them with LPS. Consistent with previous reports (*1*, *7*), poly(I:C)-primed *Casp11*$^{-/-}$ mice and *Gsdmd*$^{I105N/I105N}$ mice (which bear a GSDMD cleavage site mutation that renders the protein resistant to proteolytic activation by caspase-1 or caspase-11) were more resistant to LPS challenge than *Tlr4*$^{-/-}$ mice, supporting the idea that the activation of caspase-11–mediated GSDMD cleavage (but not TLR4) is essential for cytosolic LPS-induced endotoxic shock (Fig. 6A). Using this caspase-11–dependent sepsis mouse model, we further observed that systemic administration of L-adrenaline, 8-Br-cAMP, or PF-04957325 rescued poly(I:C)-primed mice from LPS lethality (Fig. 6B). The serum levels of organ dysfunction enzymes [e.g., creatine kinase (CK), alanine aminotransferase (ALT), and blood urea nitrogen (BUN)], inflammasome cytokines (e.g., IL-1β and IL-18), and pyroptosis markers (e.g., LDH and HMGB1) were all reduced in poly(I:C)/LPS-challenged mice after treatment with L-adrenaline, 8-Br-cAMP, or PF-04957325 (Fig. 6C). In contrast, the levels of TNF (tumor necrosis factor) and IL-6 were not changed by these inhibitors (Fig. 6C). Increasing cAMP synthesis by L-adrenaline and blocking cAMP hydrolysis by the PDE8A inhibitor PF-04957325 also elevated serum cAMP levels in mice during endotoxic shock (fig. S3). Moreover, acute starvation-induced stress increased serum cAMP levels (fig. S4A) and protected against poly(I:C)/LPS-induced endotoxic shock in mice (fig. S4B). Together, these findings suggest that cAMP metabolism controls caspase-11–mediated cytokine release and tissue injury in endotoxic shock.

We also evaluated other top drug candidates from in vitro screening in an endotoxic shock model. Prednisolone and quinapril, but not levosulpiride, increased animal survival in poly(I:C)/LPS-induced endotoxic shock (Fig. 6D). Similar to L-adrenaline administration, the serum cAMP was up-regulated, whereas CK, BUN, ALT, IL-1β, IL-18, and LDH were down-regulated by prednisolone and quinapril (Fig. 6E), indicating that these drugs use different pathways to increase cAMP production in controlling caspase-11–mediated lethal endotoxic shock.

## DISCUSSION

Inflammasome formation and activation is a complex process characterized by dynamic changes in molecular assembly and metabolic activity. Compared to the canonical caspase-1 inflammasome, the more recently identified noncanonical caspase-11 inflammasome remains poorly understood in its mechanistic details and pathophysiological implications. In this study, we reveal a role for cAMP in counter-regulating caspase-11–dependent inflammasome activation in vitro and in vivo. Specifically, several cAMP-relevant receptors, enzymes, and effectors—including ADRA2B, ADCY4, PDE8A, and PKA—are involved in the control of caspase-11 inflammasome activation and pyroptosis (fig. S5). These findings may improve our understanding of the metabolic mechanisms involved in the perpetuation or resolution of inflammation (*15*).

The innate immune system detects Gram-negative bacteria partly by recognizing LPS, a typical pathogen-associated molecular pattern (*16*). The proposed mechanism of LPS recognition by macrophages generally includes two recognition systems. On the cell surface, TLR4 is required for the detection of extracellular LPS to initiate expression of a wide array of inflammatory mediators (*6*). Within the cell, caspase-11 acts as an endogenous receptor for the recognition of cytosolic LPS to induce pyroptosis (*7*–*11*). This combined intra- and extracellular action of LPS on macrophages triggers massive immune responses that not only facilitate the eradication of infectious pathogens but also cause inflammatory injury and endotoxemia if regulated improperly. Given that the application of TLR4 antagonists (e.g., Eritoran) for the treatment of patients with sepsis failed in recent clinical trials (*17*), the caspase-11 pathway has become an attractive alternative target to treat lethal infectious diseases (*18*, *19*).

In this study, we initially aimed at identifying FDA-approved drugs that block caspaspe-11–dependent pyroptosis in macrophages. Unexpectedly, one lead candidate that effectively inhibits cytosolic LPS-induced pyroptosis in macrophages is L-adrenaline, which is normally produced by the adrenal glands and certain neurons. Although L-adrenaline has been used for long as a vasoactive drug in septic shock (*20*), our current study indicates that the cytosolic LPS-induced caspase-11 inflammasome pathway is a previously unidentified target of L-adrenaline in macrophages. Among many L-adrenaline receptors on the surface of immune cells, we demonstrated that L-adrenaline abolishes caspase-11 inflammasome activation via ADRA2B, a β-adrenergic receptor expressed in various macrophage subtypes (*21*). Dopamine, another vasoactive agent used in sepsis patients, has been recently reported to inhibit NLRP3 inflammasome activation in macrophages via the dopamine receptor D1 (*22*). This evidence indicates that hormones or neurotransmitters could act as critical endogenous modulators of inflammasome activation.

We succeeded in identifying major regulatory hubs connecting L-adrenaline stimulation to the blockade of caspase-11 inflammasome. cAMP acts as the critical intracellular mediator of L-adrenaline activity in the inhibition of caspase-11 inflammasome. cAMP was first reported in 1956 as an intracellular second messenger mediating the effects of L-adrenaline and glucagon on glycogenolysis in the liver tissues (*23*). In addition to generating hormone-specific effects in the nervous system, elevations in cAMP levels also have important effects on immune cell differentiation, function, and response to infection (*24*). Although cAMP exhibits largely inhibitory effects on macrophage activation (*25*, *26*), the molecular regulation of cAMP metabolism is context dependent. Previous studies showed that PDE4B-mediated cAMP hydrolysis mediates extracellular LPS-induced TLR4 activation in macrophages (*27*, *28*). Our current findings indicate that ADCY4-mediated cAMP synthesis blocks, whereas PDE8A-mediated cAMP hydrolysis promotes, cytosolic LPS-induced

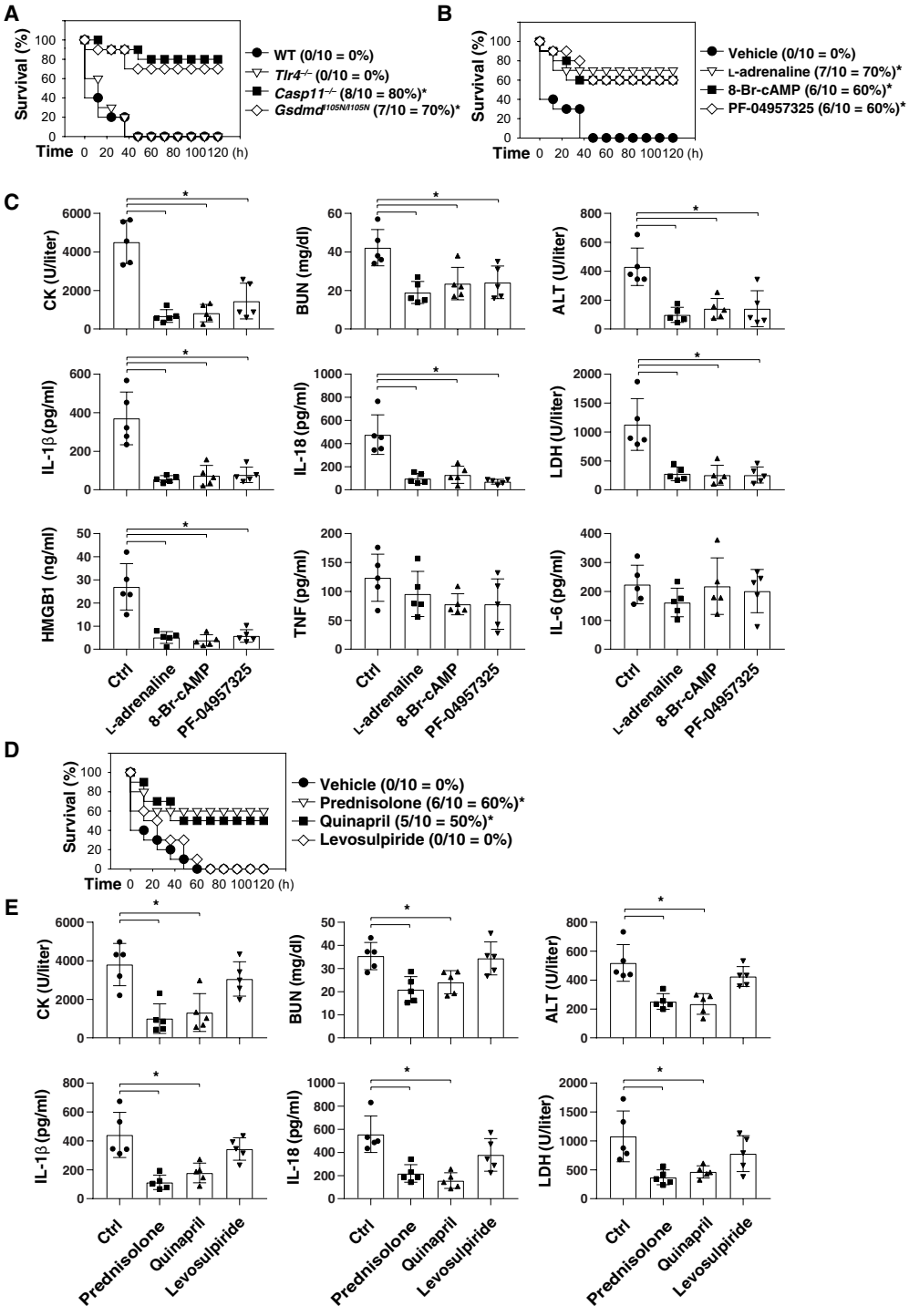


**Fig. 6. Targeting the cAMP metabolism pathway attenuates caspase-11–mediated lethal endotoxic shock.** (**A**) Survival of indicated mice primed with poly(I:C) [10 mg/kg, ip (intraperitoneally)] and then challenged 6 hours (h) later with LPS (2 mg/kg, ip) ($n = 10$ mice per group; $^*P < 0.05$, Kaplan-Meier survival analysis). (**B**) Survival of indicated mice primed with poly(I:C) (10 mg/kg, ip) and then challenged 6 hours later with LPS (2 mg/kg, ip) in the absence or presence of administration of L-adrenaline (2 mg/kg, ip), 8-Br-cAMP (10 mg/kg, ip), or PF-04957325 (5 mg/kg, ip) at −0.5, +12, +24, +36, and +48 hours ($n = 10$ mice per group; $^*P < 0.05$, Kaplan-Meier survival analysis). (**C**) In parallel to (B), quantitation of indicated serum markers in poly(I:C)-primed mice challenged with LPS in the absence or presence of L-adrenaline, 8-Br-cAMP, or PF-04957325 at +3 hours [$n = 5$ mice per group; $^*P < 0.05$, analysis of variance (ANOVA) least significant difference (LSD) test]. (**D**) Survival of indicated mice primed with poly(I:C) (10 mg/kg, ip) and then challenged 6 hours later with LPS (2 mg/kg, ip) in the absence or presence of administration of prednisolone (5 mg/kg, ip), quinapril (30 mg/kg, ip), or levosulpiride (20 mg/kg, ip) at −0.5, +12, +24, +36, and +48 hours ($n = 10$ mice per group; $^*P < 0.05$, Kaplan-Meier survival analysis). (**E**) In parallel to (D), quantitation of indicated serum markers in poly(I:C)-primed mice challenged with LPS in the absence or presence of prednisolone, quinapril, or levosulpiride at +3 hours ($n = 5$ mice per group; $^*P < 0.05$, ANOVA LSD test).

caspase-11 inflammasome activation in macrophages. Thus, both the synthesis and hydrolysis reactions of cAMP can determine the degree of caspase-11 inflammasome activation in macrophages.

We further demonstrated that PKA, but not RAPGEF3, is a major cAMP effector to block caspase-11 inflammasome activation in macrophages. PKA is composed of catalytic and regulatory subunits (*29*). In the absence of cAMP, PKA exists in a catalytically inactive form bound to the regulatory subunit (*29*). Our results suggest that the activation of PKA by L-adrenaline depends on cAMP, which inhibits caspase-11 activity and IL-1β release in response to cytosolic LPS. In addition to the noncanonical inflammasome, cAMP can also shut down canonical inflammasome activation and restrain inflammation (*30*, *31*). Recent studies have shown that the activation of PKA by bile acids or prostaglandin E2 inhibits NLRP3 inflammasome activation through directly phosphorylating NLRP3 in macrophages (*32*, *33*). PKA phosphorylates numerous metabolic enzymes, which may provide further feedback control of inflammasome activation (*34*). We found that PKA can bind caspase-11 to promote caspase-11 phosphorylation, which may contribute to the inhibition of caspase-11 activation. RAPGEF3, but not PKA, is required for cAMP-mediated NLRP3 inflammasome inhibition in endothelial cells (*35*). Thus, distinct, perhaps cell type–specific cAMP effector molecules may contribute to the regulation of inflammasome.

In summary, our in vitro results demonstrate that L-adrenaline inhibits caspase-11 inflammasome activation through the ADRA2B receptor and the intracellular cAMP metabolism pathway in mouse macrophages or human monocytes. Our in vivo data confirm that targeting the ADRA2B-ADCY4-PDE8A-PKA axis blocks the caspase-11–mediated inflammatory response and tissue injury in septic mice. Thus, increasing cAMP levels in relevant immune cell subsets may provide an effective metabolism-based therapy for the treatment of lethal infection. Notably, the PKA signaling involved the association of PKA with a family of scaffolding proteins termed A kinase–anchoring proteins (AKAPs), which included 70 functionally distinct members (*36*). It remains of great interest to know which classes of AKAP can modulate PKA activity in the inhibition of caspase-11 activation in the future.

## MATERIALS AND METHODS

Reagents are described in table S1.

### Cell culture and bacterial infection

The THP1 (no. TIB-202) cell line was obtained from the American Type Culture Collection (ATCC). Immortalized BMDMs from wild-type, *Nlrp3*$^{-/-}$, *Nlrc4*$^{-/-}$, and *Nlrp1*$^{-/-}$ mice were a gift from K. Fitzgerald (University of Massachusetts Medical School). BMDMs from *Casp11*$^{-/-}$ mice were obtained using 30% L929 cell–conditioned medium as a source of granulocyte/macrophage colony stimulating factor. CRISPR-Cas9–mediated *Gsdmd*$^{-/-}$ BMDMs were a gift from D. Abbott (Case Western Reserve University). Human peripheral blood monocytes were obtained from STEMCELL Technologies (no. 70034). These cells were cultured in Dulbecco's modified Eagle's medium (DMEM; no. 11995073, Thermo Fisher Scientific) supplemented with 10% heat-inactivated fetal bovine serum (no. TMS-013-B, Millipore) and 1% penicillin and streptomycin (no. 15070-063, Thermo Fisher Scientific) at 37°C, 95% humidity, and 5% $CO_2$. Cells were primed with LPS (200 ng/ml, 6 hours), poly(I:C) (5 μg/ml, 6 hours), or Pam3CSK4 (1 μg/ml, 6 hours) and then stimulated with LPS electroporation (1 μg, 16 hours) or *E. coli* [multiplicity of infection (MOI), 25; 16 hours] infection (*5*, *37*, *38*). All cells used were authenticated using short tandem repeat (STR) profiling, and mycoplasma testing was negative.

*E. coli* (no. 11775) was obtained from the ATCC and then added to cells at an MOI of 25 in medium without antibiotics. After 30 min, cells were washed and incubated for 1.5 hours at 37°C in fresh medium supplemented with gentamicin (100 μg/ml; no. G1397, Sigma-Aldrich) to kill extracellular bacteria.

### Animal model of septic shock

We conducted all animal care and experiments in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care guidelines (www.aaalac.org) and with approval from our institutional animal care and use committee. Mice were housed with their littermates in groups of four or five animals per cage and kept on a regular 12-hour light and dark cycle in a specific pathogen-free barrier facility.

*Gsdmd*$^{I105N/I105N}$ mice (C57BL/6) were a gift from V. M. Dixit (Genentech Inc.). *Casp11*$^{-/-}$ mice (C57BL/6) were a gift from T. R. Billiar (University of Pittsburgh). *Tlr4*$^{-/-}$ (C57BL/6, no. 007227) mice were obtained from the Jackson Laboratory.

Septic shock was induced in male or female C57BL/6 mice (8 to 10 weeks old, 22- to 26-g body weight). These mice were primed with poly(I:C) [10 mg/kg, ip (intraperitoneally)] and then challenged 6 hours later with LPS (2 mg/kg, ip) (*7*). At 30 min, before the second LPS injection, mice were subcutaneously injected with inhibitors [L-adrenaline (2 mg/kg, ip), 8-Br-cAMP (10 mg/kg, ip), PF-04957325 (5 mg/kg, ip), prednisolone (5 mg/kg, ip), quinapril (30 mg/kg, ip), or levosulpiride (20 mg/kg, ip)]. For each of the next 2 days, these inhibitors were injected at 12-hour intervals.

For starvation, mice were placed in clean cages without food for 48 hours. Water was given ad libitum. After starvation, these mice were primed with poly(I:C) (10 mg/kg, ip) and then challenged 6 hours later with LPS (2 mg/kg, ip under normal diet.

### Cell viability assay

Cells were seeded into 96-well plates and incubated with the indicated treatments. Subsequently, 100 μl of fresh medium was added to cells containing 10 μl of Cell Counting Kit-8 (CCK-8) solutions (CK04, Dojindo Laboratories) and incubated for 2 hours (37°C, 5% $CO_2$). Absorbance at 450 nm was measured using a microplate from BioTek.

### Biochemical assay

Commercially available enzyme-linked immunosorbent assay kits were used to measure the concentrations or activity of HMGB1 (no. ST51011, Shino-Test Corporation), IL-1β (no. MLB00C or no. DLB50, R&D Systems), IL-18 (no. 7625, R&D Systems), LDH (no. ab102526, Abcam), IL-6 (no. M6000B, R&D Systems), TNF (no. MTA00B, R&D Systems), cAMP (no. ADI-901-067, Enzo Life Sciences), and PKA (no. ab139435, Abcam) in indicated samples. Measurement of serum tissue enzymes (CK, BUN, and ALT) was performed using the IDEXX Catalyst Dx Chemistry Analyzer.

### LPS transfection

To stimulate caspase-11 noncanonical inflammasome activation, LPS was electroporated into indicated cells using the Neon Transfection System (Thermo Fisher Scientific) according to the manufacturer's protocol. Briefly, BMDMs were electroporated with LPS

(1 to 3 μg) in buffer R (no. MPK10025, Thermo Fisher Scientific) under pulse voltage of 1400 V, pulse width of 10 ms, and pulse number 2 (*5*).

### RNA interference

ON-TARGETplus SMARTpool siRNAs against indicated genes as described in table S1 were purchased from Dharmacon. This pool was a mixture of four siRNAs provided as a single reagent. The Neon Electroporation System from Invitrogen was used to deliver siRNAs into BMDMs. Transfected cells were recovered in complete DMEM. The medium was replaced at 3 hours after electroporation. The cells were cultured for 48 hours before further examination.

### Western blot

Western blot was used to analyze protein expression as described previously (*39*). Briefly, after extraction, proteins in cell lysates were first resolved by 4 to 12% Criterion XT bis-tris gel electrophoresis and then transferred to a polyvinylidene difluoride membrane and subsequently incubated with the primary antibody (1:500 to 1:1000). After incubation with peroxidase-conjugated secondary antibodies (1:1000 to 1:2000), the signals were visualized by enhanced chemiluminescence (no. 32106, Thermo Fisher Scientific) according to the manufacturer's instructions.

### Quantitative real-time polymerase chain reaction

Total RNA was extracted using TRI reagent (no. 93289, Sigma-Aldrich) according to the manufacturer's instructions. First-strand complementary DNA (cDNA) was synthesized from 1 μg of RNA using the iScript cDNA Synthesis Kit (no. 1708890, Bio-Rad). cDNA from various cell samples was amplified using real-time qPCR with specific primers as described in table S1. The data were normalized to 18*S* RNA, and the fold change was calculated via the $2^{-\Delta\Delta Ct}$ method (*40*). Relative concentrations of mRNA were expressed in arbitrary units based on the untreated group, which was assigned a value of 1.

### Immunoprecipitation analysis

Cells were lysed at 4°C in ice-cold radioimmunoprecipitation assay lysis buffer, and cell lysates were cleared by brief centrifugation (12,000*g* for 10 min). Concentrations of proteins in the supernatant were determined by bicinchoninic acid (BCA) assay. Before immunoprecipitation, samples containing equal amounts of proteins were precleared with protein G agarose (4°C for 3 hours) and subsequently incubated with various irrelevant immunoglobulin G or anti–caspase-11 antibodies (4 μg/ml) in the presence of protein G agarose beads for 2 hours or overnight at 4°C with gentle shaking. Following incubation, agarose beads were washed extensively with phosphate-buffered saline, and proteins were eluted by boiling in 2× SDS sample buffer before SDS–polyacrylamide gel electrophoresis for measurement of caspase-11, PRKACA, or p-Ser.

### Statistical analysis

Data are presented as means ± SD. All data meet the assumptions of the tests (e.g., normal distribution). Unpaired Student's *t* tests were used to compare the means of two groups. One-way analysis of variance (ANOVA) was used for comparison among the different groups. When ANOVA was significant, post hoc testing of differences between groups was performed using the least significant difference test. The Kaplan-Meier method was used to compare differences in mortality rates between groups. A $P < 0.05$ was considered statistically significant. The exact value of *n* within figures was indicated in the figure legends. We did not exclude samples or animals. For every figure, statistical tests are justified as appropriate. All data meet the assumptions of the tests. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those generally used in the field.

## SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/5/5/eaav5562/DC1

Fig. S1. Effects of L-adrenaline on cytosolic LPS-induced pyroptosis in human primary monocytes.

Fig. S2. Effects of L-adrenaline on LPS priming in BMDMs.

Fig. S3. Effects of L-adrenaline and PF-04957325 on serum cAMP level in mice with endotoxic shock.

Fig. S4. Effects of acute starvation-induced stress on endotoxic shock.

Fig. S5. Schematic summary of cAMP metabolism in the regulation of cytosolic LPS-induced caspase-11 inflammasome activation and pyroptosis.

Table S1. Reagent sources.

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