Distinct regions of NLRP1B are required to respond to anthrax lethal toxin and metabolic inhibition

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Running title: Distinct sensor regions of NLRP1B

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SUMMARY

Pattern recognition receptors monitor for signs of infection or cellular dysfunction and respond to these events by initiating an immune response. NLRP1B is a receptor that upon activation recruits multiple copies of pro-caspase-1, which promotes cytokine processing and a pro-inflammatory form of cell death termed pyroptosis. NLRP1B detects anthrax lethal toxin when the toxin cleaves an amino-terminal fragment from the protein. In addition, NLRP1B is activated when cells are deprived of glucose or treated with metabolic inhibitors, but the mechanism by which the resulting reduction in cytosolic ATP is sensed by NLRP1B is unknown. Here, we have addressed whether these two activating signals of NLRP1B converge on a common sensing system. We show that an NLRP1B mutant lacking the amino-terminal region exhibits some spontaneous activity and fails to be further activated by lethal toxin. This mutant was still activated in cells depleted of ATP, however, indicating that the amino-terminal region is not the sole sensing domain of NLRP1B. Mutagenesis of the leucine rich repeat domain of NLRP1B provided evidence that this domain is involved in auto-inhibition of the receptor, but none of the mutants tested was specifically defective at sensing activating signals. Comparison of two alleles of NLRP1B that differed in their response to metabolic inhibitors, but not to lethal toxin, led to the finding that a repeated sequence in the FIIND domain that arose from exon duplication facilitated detection of ATP depletion. These results suggest that distinct regions of NLRP1B detect activating signals.
INTRODUCTION

Inflammasomes are multi-protein complexes that activate pro-caspase-1 in response to pathogen-associated molecular patterns (PAMPS) and danger-associated molecular patterns (DAMPS) (1). Activated caspase-1 processes pro-inflammatory cytokines IL-1β and IL-18 and causes cells to undergo a form of death known as pyroptosis. Pyroptosis is important for the elimination of compromised cells and for the recruitment of immune cells to the site of infection or injury (2). Secretion of IL-1β and IL-18 promotes recruitment and activation of inflammatory cells and leads to further production of cytokines important for stimulation of the immune response.

NLRP1B is a pattern recognition receptor that triggers caspase-1 processing after it detects an activating signal. NLRP1B contains a NACHT domain (domain present in NAIP, CIITA, HET-E, TP-1), a central leucine rich repeat domain (LRR), a ‘function to find’ domain (FIIND), and a carboxy-terminal caspase recruitment domain (CARD) (3, 4). The NACHT domain is a nucleotide-binding domain that is involved in self-association of NLRP1B (5). The LRR domain has been predicted to be involved in ligand recognition and in intra-molecular interactions that mediate auto-inhibition (1, 4, 6). The FIIND domain undergoes an auto-proteolytic event, which facilitates inflammasome assembly (7-9) and the CARD domain of NLRP1B interacts directly with the CARD domain of pro-caspase-1 (6).

NLRP1B was originally shown to be activated by anthrax lethal toxin (LeTx) (3). LeTx, a binary toxin composed of protective antigen (PA) and lethal factor (LF), is secreted by the bacterium *Bacillus anthracis* during infection (10). PA binds to host cellular receptors and translocates the protease LF into the cytosol. Recent studies
demonstrated that LF cleaves the amino-terminus of NLRP1B and that this cleavage is sufficient for activation (11-13).

Reduction in cytosolic ATP is a second activator of NLRP1B (14). This signal might allow NLRP1B to sense when the cell has entered damaged tissue that is low in glucose and oxygen. Additionally, monitoring cytosolic ATP might enable cells to detect intracellular pathogens that diminish nucleotide pools. Recently, the intracellular parasite *Toxoplasma gondii* was found to induce NLRP1B inflammasome assembly (15-17), although the activation signal elicited by *Toxoplasma* was not determined and may be distinct from ATP depletion.

It is unclear how NLRP1B detects the reduction of cellular ATP or if this signal is direct or indirect. It has been suggested that the amino-terminal region of NLRP1B may serve as a sensor domain, not only for LF proteolytic activity, but for a variety of pathogen proteases (11). We wanted to address whether the amino-terminal region of NLRP1B also serves as a sensor of reduced ATP levels or if a distinct region of NLRP1B is involved. By comparing the abilities of LeTx and ATP depletion to activate a series of NLRP1B mutants, we confirmed that the amino-terminal region is required for sensing LeTx and found that this region is dispensable for the detection of ATP reduction. We isolated LRR mutants that were spontaneously active, but none that was non-responsive to activating signals. We did, however, engineer deletion mutants within the FIIND that were not fully activated by ATP depletion, suggesting that this domain contributes to signal sensing.
MATERIALS AND METHODS

Cell culture and reagents.

HT1080 cells (ATCC) were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. Protective antigen (PA) and lethal factor (LF) were purified as described previously, and applied to cells at a final concentration of 10^{-8} M (18). Sodium azide (NaN_3) and 2-deoxyglucose (2DG) were purchased from Sigma-Aldrich and used at the indicated concentrations.

Plasmid construction and site-directed mutagenesis.

Plasmids encoding NTAP-NLRP1B allele 1, procaspase-1-T7, and pro-IL-1β-hemagglutinin (HA) have been described previously (6).

QuikChange site-directed mutagenesis (Stratagene) was performed according to the manufacturer’s instructions to generate pNTAP-NLRP1B_{43-QAQ-45}, pNTAP-NLRP1B LRR mutants and pNTAP-NLRP1B allele 5 (for primer sequences see Table S1). pcDNA3-NLRP1B-3XFlag was generated by amplifying NLRP1B from NTAP-NLRP1B allele 1 using the forward primer NLRP1BFor and the reverse primer NLRP1BRev. The PCR product was digested with BamHI and XhoI and then ligated into pcDNA3XFlag.

NLRP1B N-terminal truncation plasmids were constructed by amplifying fragments from pcDNA3-NLRP1B-3XFlag allele 1. The reverse primer FlagRev was used with the forward primers NLRP1B40F, NLRP1B45F, NLRP1B50F, NLRP1B60F, NLRP1B70F and NLRP1B80F to amplify the designated fragments, NLRP1B_{40-1233}, NLRP1B_{45-1233}, NLRP1B_{50-1233}, NLRP1B_{60-1233}, NLRP1B_{70-1233}, and NLRP1B_{80-1233}. The
PCR products were digested with BamHI and NheI and then ligated into pcDNA3.

To construct pNTAP-NLRP1B\(_{\Delta810-870}\), pNTAP-Nlrp1\(_{\Delta749-809}\) and pNTAP-Nlrp1\(_{\Delta749-870}\), standard molecular biology techniques were used. To construct pNTAP-Nlrp1\(_{\Delta749-809}\) and pNTAP-Nlrp1\(_{\Delta749-870}\), an Ascl site was introduced into pNTAP-NLRP1B allele 1 by using the NLRP1BAscl primer and its complement. The plasmid was digested with Ascl and used as template for PCR. The reverse primer NLRP1BFIINDdelR was used with the forward primers NLRP1BFIINDdel1F and NLRP1BFIINDdel2F to amplify the designated fragments pNTAP-Nlrp1\(_{\Delta749-809}\) and pNTAP-Nlrp1\(_{\Delta749-870}\). The PCR products with the designated deletions were ligated together.

**IL-1β release assay.**

One million HT1080 cells were seeded in a 10-cm dish the day before transfection. On the day of transfection, 1 \(\mu\)g each of pNTAP-NLRP1B or indicated mutant, pcDNA3–procaspase-1-T7, and pcDNA3–pro-IL-1β-HA were transfected using 9 \(\mu\)l of 1 mg/ml polyethyleneimine, pH 7.2. Approximately 24 h after transfection, cells were washed with PBS (unless stated otherwise) and then treated with LF (10^{-8} M) and PA (10^{-8} M) or 50 mM 2DG and 10 mM NaN\(_3\) for 3 h. The cell supernatant was mixed with 50 \(\mu\)l of α-HA antibody for 2 h, followed by the addition of 75 \(\mu\)l of protein A Sepharose (RepliGen) and overnight incubation. Proteins were eluted from the protein A Sepharose beads with SDS loading dye and subjected to immunoblotting using a polyclonal α-HA antibody (Santa Cruz sc805).

**RESULTS**

The amino-terminus of NLRP1B is not essential for inflammasome activation in
We demonstrated previously that metabolic inhibitors activate the NLRP1B inflammasome by using a reconstituted system (14). HT1080 human fibroblasts were transfected with plasmids encoding NLRP1B allele 1, pro-caspase-1 and pro-IL-1β and were treated with a glycolysis inhibitor, 2DG, and a mitochondrial electron transport chain inhibitor, NaN3. Treatment with these inhibitors resulted in reduction in cytosolic ATP levels and secretion of processed IL-1β. The mechanism of activation of the NLRP1B inflammasome by these metabolic inhibitors, however, is unknown. Although the amino-terminus of NLRP1B is cleaved by LF and this cleavage is sufficient for the activation of the inflammasome (11-13), we were previously unable to detect cleavage of NLRP1B in response to treatment with 2DG/NaN3 (14). Because it is conceivable that reduced ATP levels led to a small amount of NLRP1B cleavage that was below detection limits, we sought to determine whether an intact LF cleavage site is essential for 2DG/NaN3-induced activation of NLRP1B. Two LF cleavage sites have been identified in murine NLRP1B, cleavage site-1 after lysine 38 and cleavage site-2 after lysine 44 (11, 12). Cleavage site-2 was determined to be the predominant LF cleavage site since mutation of this site abolished NLRP1B activation in response to LeTx. We mutated cleavage site-2 by substituting amino acids 43LKL45 for QAQ as described previously (11) and tested the mutant for inflammasome activation. In agreement with previous reports, the mutation of LF cleavage site-2 abolished processing and release of IL-1β in response to LeTx (Fig. 1A). This mutant was still able to induce IL-1β secretion in response to 2DG/NaN3 (Fig. 1A), however, suggesting that the cleavage site was not important for NLRP1B activation by these inhibitors.
We next sought to determine whether the amino-terminus of NLRP1B is essential for this process. We first constructed an amino-terminal deletion mutant, NLRP1B_{45-1233}, that mimics LF-cleaved NLRP1B. A low level of IL-1β secretion was observed from cells expressing NLRP1B_{45-1233} in the absence of signal, indicating a degree of spontaneous activity (Fig. 1B). LeTx treatment did not induce additional IL-1β secretion (Fig. 1B). In contrast, 2DG/NaN₃ induced IL-1β secretion from cells expressing NLRP1B_{45-1233} at a comparable level as cells expressing wild-type NLRP1B (Fig. 1B). These results indicate that the “pre-cleaved” NLRP1B_{45-1233} mutant has some spontaneous activity but is still responsive to 2DG/NaN₃.

We next constructed a series of deletion mutants of NLRP1B to assess the role of the amino-terminus in signal detection. Deletion of 40 residues from the amino-terminus had little effect on IL-1β release in response to LeTx or 2DG/NaN₃ as compared to full-length NLRP1B (Fig. 1C). Deletion of between 50 and 80 amino acids, however, resulted in low spontaneous activity, non-responsiveness to LeTx, and a higher responsiveness to 2DG/NaN₃ (Fig. 1C). Altogether these results suggest that the amino-terminus of NLRP1B is essential for inflammasome activation by LeTx, but is dispensable for activation in response to metabolic inhibitors.

The LRR domain is involved in auto-inhibition of NLRP1B

It has been speculated that the leucine rich repeat (LRR) domain of NLRP1B is involved in auto-inhibition and ligand recognition (1, 4, 6): the inactive form of NLRP1B might be stabilized by an interaction between the LRR and the NACHT domains, which is relieved upon recognition of a ligand by the LRR domain. To study the LRR domain of
NLRP1B, the Phyre2 protein structure prediction server (19) was used to generate a model (residues 626-751) using structures of known LRR containing proteins (Fig. 2A). The predicted structure was based on the crystal structure of the tropomodulin LRR domain (1IO0); 99% of the sequence was modeled with 99.8% confidence. The LRR domain model exhibits a typical arch shape comprising four 28-residue repeats (Fig. 2A). Five α-helices form the convex surface and four β-strands compose the concave surface of the LRR. Typically, the β-strands that make up the concave surface of the LRR are involved in protein-protein interactions, but several LRR containing proteins have been shown to engage in ligand binding at the convex surface (20). For this reason, we mutated amino acids on both the convex and concave surfaces.

Since we suspected that the LRR domain is involved in auto-inhibition, we first analyzed all the LRR mutants for spontaneous activity in the absence of signal by assaying overnight supernatants of transfected cells for processed IL-1β (Fig. 2B and C, O/N Supernatants). We then assessed the activity of the LRR mutants in response to either LeTx or 2DG/NaN₃ by replacing overnight supernatants prior to treatment and then assaying for processed IL-1β (Fig 2B and C, Treatment Supernatants).

We found that most of the alanine substitutions at the convex surface of the LRR domain (Q644A, N648A, R651A, R670A, S673A, D698A/R701A/M702A, E705A, Q727A, T734A/K738A) had no effect on activity; only one mutant (NLRP1B-E731A) exhibited spontaneous activity and was still responsive to either LeTx or 2DG/NaN₃ (Fig. 2B). Of the eleven concave surface LRR domain mutants tested, two mutants (W661A, Y689A) behaved similarly to wild-type NLRP1B whereas the rest of the mutants demonstrated spontaneous activity and were either responsive to LeTx or 2DG/NaN₃.
(E629A, V663A/K664A, T686A/E687A, Q691A) or non-responsive to both signals
(D632A/S634A, K658A/T659A, D720A, I744A/S746A) (Fig. 2C). All mutants were
expressed at similar or slightly higher levels compared to wild-type NLRP1B in the
absence of transfected pro-caspase-1 and pro-IL-1β, suggesting that the mutations did
not destabilize the protein (Fig. S1). Co-expression of pro-caspase-1 and pro-IL-1β with
the non-responsive NLRP1B mutants consistently led to lower levels of these mutant
proteins compared to the wild-type protein, presumably because these spontaneously
active mutants were more prone to assemble into inflammasomes that were secreted or
leaked from pyroptotic cells prior to the treatments (Fig. S2). Thus, the observed non-
responsiveness of some mutants to the treatments was likely a result of low levels of
inflammasome components after the overnight transfection period. Altogether our
results suggest that it is the concave surface of the LRR domain that is predominantly
involved in the auto-inhibition of NLRP1B.

Deletions in the FIIND of NLRP1B allele 1 impair inflammasome activation in
response to metabolic inhibitors

The NLRP1B gene is polymorphic and there are 5 alleles (3). Murine
macrophages that express either allele 1 or 5 are susceptible to LeTx-induced
pyroptosis, while those that express alleles 2, 3, or 4 are resistant. We sought to
determine whether NLRP1B allele 5 responds to 2DG/NaNO₃ treatment. We established
that in the reconstituted system, LeTx induced the release of IL-1β from cells expressing
NLRP1B allele 5; the amount released was approximately 80% of that from cells
expressing NLRP1B allele 1 (Fig. 3A). Treatment of cells expressing NLRP1B allele 5
with 2DG/NaN₃ resulted in ~40% of the amount of IL-1β compared to the amount released by cells expressing allele 1 (Fig. 3A). These results suggest that NLRP1B allele 5 has a diminished capacity to be activated by metabolic inhibitors.

Alignment of NLRP1B alleles 1 and 5 reveal 13 amino acid differences and a 61 amino acid insertion in allele 1 (Fig. S3). This insertion is due to duplication of exons in a region of the gene that encodes the FIIND (3). The first repeat (encoding 61 amino acids) is missing in allele 2 and the second repeat (encoding 61 amino acids) is missing in alleles 3, 4 and 5 (Fig. 3B). To determine whether the duplication increased the responsiveness of allele 1 to the metabolic inhibitors, we created deletions in the FIIND of NLRP1B allele 1 that correspond to the deletion found in allele 2 (NLRP1BΔ749-809) and the deletion found in alleles 3 to 5 (NLRP1BΔ810-870). We found that LeTx was able to induce IL-1β secretion from cells expressing either NLRP1BΔ749-809 or NLRP1BΔ810-870 at levels similar to those from cells expressing wild-type NLRP1B allele 1 (~95% and ~90% respectively) (Fig. 3C). In contrast, NLRP1BΔ749-809 and NLRP1BΔ810-870 were attenuated in their abilities to respond to treatment with 2DG/NaN₃: approximately 20-25% of the amount of IL-1β was released compared to wild-type NLRP1B allele 1 expressing cells. This suggested the deletion of either one of the repeats affects the responsiveness of NLRP1B to the metabolic inhibitors.

To assess whether deletion of both repeats further decreased responsiveness to the metabolic inhibitors, we constructed NLRP1BΔ749-870. NLRP1BΔ749-870 exhibited no activity in response to either LeTx or 2DG/NaN₃ (Fig. 3C). Immunoblots of the deletion mutants showed that NLRP1BΔ749-870 ran as a single band on a gel instead of a double band that was observed for wild-type NLRP1B allele 1, NLRP1BΔ749-809 and NLRP1BΔ810-870.
(Fig. S4), suggesting that the deletion prevented auto-proteolysis of NLRP1B, which is important for the assembly of the inflammasome (7-9).

DISCUSSION

Sensing of LeTx by NLRP1B occurs when LF cleaves the NLRP1B amino-terminal region to relieve auto-inhibition (11, 12). It was suggested that this region of NLRP1B might serve as a detector not only for LF, but for other pathogen proteases (11). Since we previously demonstrated that reduction in cytosolic ATP also induced NLRP1B inflammasome activation (14), we wished to address whether metabolic stress was also sensed through this region, possibly as a consequence of being cleaved by mislocalized host proteases. We did not, however, detect cleavage of NLRP1B in cells depleted of ATP (14) and we found that an NLRP1B mutant lacking the entire amino-terminal region was still responsive to metabolic inhibitors (Fig. 1C). That metabolic inhibitors activate this mutant suggests that cleavage of the amino-terminus, although sufficient to induce inflammasome assembly, does not efficiently activate the protein.

Consistent with this notion, we found that cleavage of wild-type NLRP1B by LF leads to secretion of more IL-1β than spontaneously active NLRP1B45-1233, which mimics LF-cleaved NLRP1B (Fig. 1B). It is possible, therefore, that binding of LF to a region outside the amino-terminal domain contributes to NLRP1B activation. We note that LF did not further activate NLRP1B45-1233, but it could be that LF binds only very weakly to a mutant that lacks the substrate binding site. It is also conceivable that binding and cleavage must occur simultaneously for NLRP1B to be efficiently activated. Overall, this
work indicates that distinct mechanisms lead to inflammasome assembly in response to 
LF and to depletion of ATP.

NLRP1B may directly sense cytosolic ATP levels to regulate its activity. This idea 
was suggested by the finding that mutation of the Walker A motif of the NACHT domain 
results in spontaneous activity (14). A decrease in cytosolic ATP levels might lead to 
nucleotide-free NLRP1B that self-assembles into an inflammasome. Although this 
model is attractive in its simplicity, it needs to be tested experimentally using a 
combination of biochemical and physiological approaches. We thought, however, that it 
would also be informative to examine other regions of NLRP1B that might be involved in 
regulation or in detecting metabolic stress. We initially focused our attention on the 
LRR domain because LRR domains found in pattern recognition receptors play roles in 
ligand recognition and auto-inhibition (20, 21) and we previously found that deletion of 
the NLRP1B LRR domain results in spontaneous activity suggesting that it is involved in 
negative regulation (6).

Mechanistic insight into how LRR domains facilitate auto-inhibition was provided 
by a study in which the structure of auto-inhibited NLRC4 was solved (22). This study 
showed that the LRR domain makes contact with the nucleotide-binding and 
oligomerization domain to keep NLRC4 in a monomeric state; mutations that disrupted 
this interaction resulted in constitutive activation. We mapped mutations in the NLRP1B 
LRR domain that caused spontaneous activity to the concave surface of the domain. 
Although our data suggested that the LRR domain is involved in auto-inhibition, we can 
not rule out the possibility that it is also involved in ligand detection: mutation of amino
acids involved in both auto-inhibition and signal detection could yield a spontaneously
active phenotype. We then made use of allelic differences in NLRP1B to design two deletions in the
FIIND domain that decreased responsiveness of NLRP1B to metabolic stress. There
are five alleles of Nlrp1b and only two of these (alleles 1 and 5) encode proteins that are
activated by LeTx (3). We found that NLRP1B encoded by allele 5 had a diminished
capacity to be activated by 2DG/NaN3 compared to the allele 1 protein and mapped the
difference in sensitivity to a repeated sequence within the FIIND domain. Allele 1
contains both repeats, while allele 5 is missing the second repeat (Fig. 3B). This
repeated region in the allele 1 protein might detect a signal derived from metabolic
stress or indirectly affect signal sensing by another domain. Allele 2 encodes a protein
that is missing the first repeat (and has numerous amino acid differences compared to
the allele 1 protein) – it is not activated by LeTx and has not been tested for
responsiveness to metabolic stress. The allele 3 protein is defective at auto-processing
of the FIIND domain, which impairs inflammasome assembly, rendering it non-
responsive to LeTx and ATP depletion (9). The allele 4 protein lacks the CARD domain
that recruits pro-caspase-1 so it is unlikely to form a functional inflammasome.

Toxoplasma gondii infection has been shown to induce inflammasome activation
that is dependent on NLRP1B in mice and on NLRP1A in rats (15-17, 23). There are two
NLRP1 paralogs in rats (Nlrp1a and Nlrp1b) (24): the Nlrp1a paralog is associated with
rat macrophage susceptibility to LeTx (25), but little is know about the Nlrp1b paralog.
Amino-terminal cleavage of murine NLRP1B or rat NLRP1A in response to Toxoplasma
gondii has not been demonstrated and it might be that the mechanism of activation is
distinct from that of LeTx. One possibility is that a common activation signal derived from
Toxoplasma gondii infection and 2DG/NaN₃ treatment is detected by these NLRP1
homologs: cellular invasion by Toxoplasma gondii could induce metabolic stress within
the cell. Many protozoan parasites, including Toxoplasma gondii and Plasmodium
falciparum, cannot synthesize purines and must take up host purines using nucleoside
transporters (26, 27). Parasite ecto-nucleoside triphosphate diphosphohydrolases may
facilitate purine salvage and deplete host ATP so it will be interesting to learn whether
this contributes to inflammasome activation.
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Inflammasome Sensors NLRP1 and NLRP3 in Murine Resistance to Toxoplasma gondii. mBio 5.


Figure 1. The N-terminus of NLRP1B is not essential for inflammasome activation in response to metabolic inhibitors. (A) HT1080 cells expressing pro-caspase-1-T7, pro-IL-1β-HA and either wild-type NLRP1B, or LF cleavage mutant NLRP1B43-QAQ-45, were treated with LeTx or 50 mM 2DG/10 mM NaN3 for 3 h. Cell supernatants were immunoprecipitated with anti-HA antibodies and then probed for HA-tagged IL-1β by immunoblotting. (B) HT1080 cells expressing pro-caspase-1-T7, pro-IL-1β-HA and either full length NLRP1B1-1233 or NLRP1B45-1233, were treated with LeTx or 50 mM 2DG/10 mM NaN3 for 3 h. Cell supernatants were immunoprecipitated with anti-HA antibodies and then probed for HA-tagged IL-1β by immunoblotting. (C) HT1080 cells expressing pro-caspase-1-T7, pro-IL-1β-HA and with either full length NLRP1B1-1233 or with the indicated N-terminal deletion mutants of NLRP1B, were treated with LeTx or 50 mM 2DG/10 mM NaN3 for 3 h. Cell supernatants were immunoprecipitated with anti-HA antibodies and then probed for HA-tagged IL-1β by immunoblotting. Blots are representative of three independent experiments.

Figure 2. The LRR domain is involved in auto-inhibition of NLRP1B. (A) Predicted model of the LRR domain of NLRP1B generated by Phyre2 server (19) using PDB 1IOO as a template. Amino acids whose side chains were predicted to be accessible for protein-protein interactions at the convex (left panel) and the concave (right panel) surfaces are shown in red. (B) HT1080 cells were transfected with plasmids encoding pro-caspase-1-T7, pro-IL-1β-HA and either wild-type NLRP1B or indicated convex surface LRR
mutants. Approximately 24 h after transfection, cell supernatants were either
immunoprecipitated with anti-HA antibodies and then probed for HA-tagged IL-1β by
immunoblotting (left, O/N Supernatants) or were replaced with media containing LeTx or
50 mM 2DG/10 mM NaN₃ and then were immunoprecipitated with anti-HA antibodies
and then probed for HA-tagged IL-1β by immunoblotting (right, Treatment
Supernatants). (C) HT1080 cells were transfected with plasmids encoding pro-caspase-
1-T7, pro-IL-1β-HA and either wild-type NLRP1B or indicated concave surface LRR
mutants. Approximately 24 h after transfection, cell supernatants either
immunoprecipitated with anti-HA antibodies and then probed for HA-tagged IL-1β by
immunoblotting (left, O/N Supernatants) or were replaced with media containing LeTx or
50 mM 2DG/10 mM NaN₃ and then immunoprecipitated with anti-HA antibodies and then
probed for HA-tagged IL-1β by immunoblotting (right, Treatment Supernatants).
Figure 3. Deletions in the FIIND domain of NLRP1B allele 1 impair inflammasome activation in response to metabolic inhibitors. (A) HT1080 cells expressing pro-caspase-1-T7, pro-IL-1β-HA and either NLRP1B allele 1 or NLRP1B allele 5, were treated with LeTx or 50 mM 2DG/10 mM NaN₃. Cell supernatants were immunoprecipitated with anti-HA antibodies and then probed for HA-tagged IL-1β by immunoblotting. IL-1β was quantified (top) using the immunoblot analysis (bottom) from 6 different experiments. Representative experiment is shown (data represents mean and s.d. **** p<0.0001, Student’s t-test). (B) Sequence alignments of NLRP1B FIIND domains of alleles 1-5. Residues in light gray and dark gray represent conserved and non-conserved missense mutations relative to allele 1. (C) HT1080 cells expressing pro-caspase-1-T7, pro-IL-1β-HA and either wild-type NLRP1B allele 1 or NLRP1B allele 1 with the indicated deletion in the FIIND domain, were treated with LeTx or 50 mM 2DG/10 mM NaN₃. Cell supernatants were immunoprecipitated with anti-HA antibodies and then probed for HA-tagged IL-1β by immunoblotting. IL-1β was quantified (top) using the immunoblot analysis (bottom) from 7 different experiments. Representative experiment is shown (data represents mean and s.d., *** p<0.001, **** p<0.0001, ANOVA analysis with Dunnett’s post-test).