**Legionella** effector MavC targets the Ube2N-Ub conjugate for noncanonical ubiquitination

Kedar Puvar$^{1,4}$, Shalini Iyer$^{1,4}$, Jiaqi Fu$^{2}$, Sebastian Kenny$^{1}$, Kristos I. Negrón Terón$^{1}$, Zhao-Qing Luo$^{2}$, Peter S. Brzovic$^{3,4}$, Rachel E. Klevit$^{3,5}$ & Chittaranjan Das$^{1,6}$

The bacterial effector MavC modulates the host immune response by blocking Ube2N activity employing an E1-independent ubiquitin ligation, catalyzing formation of a γ-glutamyl-ε-Lys (Gln40Ub-Lys92Ube2N) isopeptide crosslink using a transglutaminase mechanism. Here we provide biochemical evidence in support of MavC targeting the activated, thioester-linked Ube2N-ubiquitin conjugate, catalyzing an intramolecular transglutamination reaction, covalently crosslinking the Ube2N and Ub subunits effectively inactivating the E2-Ub conjugate. Ubiquitin exhibits weak binding to MavC alone, but shows an increase in affinity when tethered to Ube2N in a disulfide-linked substrate that mimics the charged E2-Ub conjugate. Crystal structures of MavC in complex with the substrate mimic and crosslinked product provide insights into the reaction mechanism and underlying protein dynamics that favor transamidation over deamidation, while revealing a crucial role for the structurally unique insertion domain in substrate recognition. This work provides a structural basis of ubiquitination by transglutamination and identifies this enzyme’s true physiological substrate.

1 Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA. 2 Purdue Institute for Inflammation, Immunology and Infectious Disease and Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, USA. 3 Department of Biochemistry, University of Washington, Seattle, WA 98195, USA. 4These authors contributed equally: Kedar Puvar, Shalini Iyer, Peter S. Brzovic. ✉ email: klevit@uw.edu; cdas@purdue.edu

https://doi.org/10.1038/s41467-020-16211-x
Protein ubiquitination is a post-translational modification used by eukaryotic organisms to regulate critical cellular processes such as protein quality control, cell cycle progression, DNA repair, autophagy, and immunity. The sequential action of three enzymes, an ATP-dependent ubiquitin activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin-ligase (E3), work to covalently attach the C-terminal glycine (G76) of ubiquitin (Ub) to target proteins, usually through formation of an isopeptide bond to a lysine side chain. Despite lacking a Ub system of their own, many pathogenic bacteria have evolved enzymes or substrate adaptors with the ability to interact with the Ub-signaling system of their eukaryotic hosts, allowing them to take control of host processes and modulate them for their benefit. Usually injected into their host cytoplasm through specialized secretion systems, various bacterial effectors have been found to use an array of strategies to hijack or exploit Ub-signaling pathways. Numerous effectors have been found to function as E3 ligases that utilize the host ubiquitination machinery to target host proteins for Ub modification. Other effectors work as deubiquitinases, enzymes that cleave the isopeptide bonds that link Ub to target proteins and reverse Ub signals. Some effectors even chemically attack and disable specific components of the eukaryotic ubiquitinating machinery directly, including covalent alteration of Ub itself.

However, in recent years, our understanding of this post-translational modification has been redefined by the discovery of bacterial enzymes that catalyze Ub transfer using strategies that bypass the canonical E1–E2–E3 pathway. This was first demonstrated for the SidE family of Legionella effectors that catalyze NAD⁺-assisted phospho-riboyl linked ubiquitination of certain host targets.

Legionella pneumophila possesses a large arsenal of effectors with over 300 examples of proteins injected into the host via its Dot/Icm Type IV secretion system. These effectors are critical in allowing L. pneumophila to form a replicative niche within the host cell where it can survive and avoid host defense mechanisms. The newly discovered L. pneumophila effector MavC serves as another fascinating example of an enzyme that targets host Ub-signaling pathways, but works in a manner that is distinctly different from the eukaryotic Ub-transfer machinery. Valleau et al. reported the structure of apo-MavC and described its function as a Ub-specific deamidase that catalyzes the conversion of Ub to its Glu40 variant. Together with structural analysis of the effector, the authors concluded that MavC is a structural and functional homolog of the canonical eukaryotic E1–E2–E3 ubiquitination machinery, including covalent alteration of Ub itself.

Fig. 1 Proposed mechanisms of MavC-catalyzed reactions and constructs used for structural studies. a Proposed mechanism of ubiquitination resulting from transglutamination reaction catalyzed by MavC. Thioester formed in Step 1 may undergo either attack by the amino group of Ube2N Lys92 (leading to formation of Ub–Ube2N) or hydrolysis (formation of deamidated Ub). Key residues from MavC in burgundy, Ube2N in light green, and Ub in teal are represented. b Diagram depicting protein constructs used for crystallization studies and NMR experiments and location of the MavC insertion domain (residues 128–225), with diagrams of Ube2N-Ub, Ube2N-SS-Ub (MavC substrates), and Ub-Ube2N (MavC product) provided for comparison.

The activity of MavC is antagonized by MvC, a protein of 50% identity with MavC that functions to remove Ub from Ub–Ube2N in later phases of infection, which points to the importance of temporal regulation of the activity of this E2 enzyme during L. pneumophila infection. Thus, MavC catalyzes what seems to be the only known example of a Ub transfer reaction that does not require a nucleotide cofactor to activate Ub prior to substrate modification.

A key assumption of the previous work was that MavC recognizes and joins free Ub and Ube2N together. However, in cells it has been predicted that E2s exist predominantly as the activated E2–Ub conjugate poised to transfer Ub to substrates. Moreover, while MavC-catalyzed Ub deamidation occurs fairly slowly compared to the transglutaminase-mediated E2 ubiquitination activity, it remains unclear how the enzyme prioritizes one activity over the other given their mutual exclusivity. Here, we demonstrate that MavC actually targets the Ube2N–Ub conjugate to catalyze an intramolecular transglutamination reaction. We present a series of crystal structures of MavC in complex with a disulfide-linked substrate that mimics the charged Ube2N–Ub conjugate and with the transglutaminase crosslinked product.
Numerous CSPs are observed upon addition of C74A-MavC1 complex in cell extracts. Our BLI measurements yielded a $K_d \approx 2.5 \mu M$ for MavC-Ube2N conjugate (Supplementary Fig. 2g). These observations suggest that free Ub binds only weakly to MavC and binding constants could not be determined with high confidence (Fig. 2a).

Though an approximate $K_d \approx 170 \mu M$ for binding of free Ub to MavC was deduced from BLI measurements, reaction assays and NMR experiments suggest the affinity is much weaker. Reaction assays monitoring Ub deamidation as a function of Ub concentration failed to saturate activity even at 375 $\mu M$ Ub, suggesting that the $K_d$ for free Ub in this reaction is in excess of 200 $\mu M$ (Fig. 2b, Supplementary Fig. 2a, b). NMR titration of $\text{Ub}^{15N}$-Ube2N using a catalytic Cys-to-Ala mutant of MavC (C74A-MavC1–384) revealed almost no chemical shift perturbations (CSPs) in the Ub spectrum at equimolar (150 $\mu M$) concentrations. Further addition of 150 $\mu M$ Ube2N to the NMR sample did not appreciably enhance the weak interaction between MavC and Ub (Fig. 2c). We note that a previous study conducting similar NMR titration experiments, but using catalytically active MavC, reported significant CSPs in Ub upon addition of MavC21. However, we found those results consistent with the MavC-catalyzed conversion of UbWT to the deamidated product, UbD404S, and not with direct protein–protein interactions (Supplementary Fig. 2c, d).

In contrast to Ub, Ube2N binds MavC with much higher affinity. Indeed, previous work identified a MavC/Ube2N complex in cell extracts21. Our BLI measurements yielded a $K_d \approx 2.5 \mu M$ for Ube2N (Fig. 2a). In NMR titration experiments, numerous CSPs are observed upon addition of C74A-MavC1–384 to $\text{Ub}^{2H,15N}$-labeled Ube2N (Supplementary Fig. 2e). The observed CSPs define a MavC recognition surface on Ube2N formed by residues in Helix1, Loop4, and Loop7 (Supplementary Fig. 2f). This is the same Ube2N surface shown to interact with numerous eukaryotic E3 ligases28. An analogous NMR titration using purified MavC insertion domain (MavC128–223) yielded Ube2N spectral changes that are remarkably similar to those observed with C74A-MavC1–384 despite the large molecular weight differences (Supplementary Fig. 2g). These observations suggest that the MavC insertion domain is primarily responsible for binding Ube2N, a conclusion supported by similar $K_d$ values obtained from BLI measurements of Ube2N binding to MavC or the MavC insertion domain (Fig. 2a, Supplementary Fig. 1e).

Despite the relatively high affinity for Ube2N, the weak interaction between MavC and Ub ($K_M > 200 \mu M$) indicates an intermolecular transglutaminase reaction between free Ub and Ube2N would be unlikely to occur under cellular conditions. As most cellular E2s are predicted to have Ub tethered to its active-site Cys via a thioester linkage27,29, we set out to assess whether Ube2N–Ub is the relevant substrate for MavC. Our approach utilized a stable Ube2N–Ub mimic30 in which the G76CUb mutant is disulfide linked to the active site Cys87Ube2N (Fig. 1b). This mimic provided greater control of reaction components and minimized experimental complications that would arise from hydrolysis of purified wild-type Ube2N–Ub during MavC reaction assays and during NMR experiments. (Hereafter, this substrate surrogate is referred to as Ube2N-SS-Ub or the disulfide conjugate.)

BLI and NMR experiments show MavC readily binds Ube2N-SS-Ub. BLI-binding titrations yield a $K_d \approx 2.4 \mu M$ for Ube2N-SS-Ub, nearly the same affinity as observed for free Ube2N (Fig. 2a), consistent with binding dominated by interactions with Ube2N. NMR experiments were performed using a disulfide conjugate in which only the Ub subunit was isotopically labeled (Ube2N-SS–$\text{2H,15N}$-Ub). In the conjugate, not all Ub resonances are observed or of equal intensity. This is due to the Ub subunit alternating between open states, where Ub makes few contacts with the E2, and closed states, where the Ub subunit is in close contact with the E2 (ref. 31). This equilibrium results in exchange broadening of resonances whose environments differ in the open and closed states. Ub resonances that remain and largely overlap with those of free Ub can be assigned by inspection (Supplementary Fig. 2h, i).

In marked contrast to the addition of MavC to free Ub (Fig. 2c), large perturbations in the spectrum of the Ub subunit of the Ube2N subunit of $\text{2H,15N}$-Ub-SS-Ube2N are now observed upon formation of an $\text{2H,15N}$-Ub-SS-Ube2N/MavC complex (Fig. 2d). An overall loss in peak intensity is observed consistent with the large increase in molecular weight (~65 kDa) upon complex formation. In addition, a number of Ub resonances disappear. Again, this behavior can be attributed to resonance exchange broadening where a subset of Ub residues exchange between contacts with MavC, Ube2N, and/or solvent. Thus, in solution, the Ub subunit is not rigidly tied to the enzyme active. However, the high local concentration of Ub provided by MavC binding of the Ube2N–Ub conjugate significantly increases observed contacts.

In targeting Ube2N–Ub for modification, MavC could catalyze deamidation of the Ub subunit, an intramolecular transglutaminase reaction between the E2 and Ub subunits, or some combination of the two. In all scenarios, tethering the C-terminus of Ub to the E2 active site must not hinder the ability of MavC to form an obligate thioester intermediate with the Glu40 side chain of the Ub subunit. Furthermore, to catalyze transglutamination, MavC must be able to orient Lys92Ube2N and Glu40Ub of the E2–Ub conjugate in proximity to form an isopeptide bond. To investigate these possibilities, we conducted assays using 25 $\mu M$ Ube2N-SS-Ub conjugate as substrate and enzymatic amounts of MavC (5 nM). Under these conditions, MavC exhibits robust transglutaminase cross-linking activity, while deamidation of Ub of the disulfide substrate was not detected (Fig. 2e, Supplementary Fig. 2k). In sharp contrast, reactions using free Ub and Ube2N at the same subunit concentrations as the disulfide conjugate (25 $\mu M$) produced no detectable transglutaminase product (Fig. 2e). These results strongly argue that MavC targets the Ube2N–Ub conjugate to catalyze an intramolecular transglutaminase reaction resulting in the formation of an isopeptide bond between Ube2N and Ub. These results are also in line with observations of Gan et al.22, which show that a mutant of Ub lacking the last two glycines is modified to a significantly lower extent than wild-type Ub.

Structural basis of transglutaminase-mediated ubiquitination. MavC-catalyzed transglutamination proceeds via an obligate thioester-linked intermediate to form an isopeptide bond between Glu40Ub and Lys92Ube2N (Fig. 1a). To gain structural insights into the mechanism underlying this noncanonical ubiquitination, we sought to crystallize MavC with both substrate and product. For crystallization trials, we used a truncated MavC construct, MavC1–384 (Fig. 1b), that otherwise retains full enzymatic activity and Ube2N binding (Supplementary Fig. 1d, e). In addition, the MavC active site residue, Cys74, was mutated to Ala (C74A) to...
prevent any modification of Ub or Ube2N during crystallization. The MavC/substrate complex was generated using the substrate mimic Ube2N-SS-Ub used in NMR studies and in biochemical assays (see Fig. 2). The substrate complex crystallized in three different space groups (Table S1), C222, R3, and P65. The product complex was generated using Ub–Ube2N product isolated from a MavC-catalyzed reaction mixture where free Ube2N and Ub were linked via a γ-glutamyl-ε-Lys isopeptide link.

The structure of MavC1–384 in all complexes (Fig. 3) is topologically identical to that in the previously determined structure of apo-MavC1-384 (PDB id 5TSC21). Architecturally, MavC1–384 is composed of three distinct lobes: a core globular domain (CG) flanked by an insertion domain (residues 128–225) on one end and an α-helical extension (HE: residues 33–66, 356–384) on its opposite end. The overall structure of MavC1–384 can be described as a C-shaped crescent with a crevice where the catalytic center is located (Fig. 3). Looking down into the concave face of the crescent, the MavC catalytic triad (residues Cys74(C74A), His231, and Gln252) is located near the center of the C-shaped structure (Fig. 3). In all structures, the Ube2N and Ub subunit bind in an extended conformation, with Ube2N on one side of the active site and Ub on the other. The Ub subunit binds between the insertion domain and the HE, positioning the Gln40Ub side chain within the MavC catalytic cleft. The structures

Table 2

| Complex            | k_on (mM−1s−1) | k_off (s−1) | k_M (µM) | k_cat (s−1) |
|--------------------|----------------|-------------|----------|-------------|
| MavC–Ub            | 40.9 ± 0.02    | 0.0982 ± 0.0061 | 2.4 ± 0.31 |
| MavC–Ube2N         | 0.044 ± 0.046  | 0.0075 ± 0.0004 | 170 ± 9.5  |
| MavC–Ub–SS–Ube2N   | 132 ± 0.04     | 0.335 ± 0.018  | 2.5 ± 0.27 |

Fig. 2 Ube2N-SS-Ub is a significantly better substrate for MavC than free Ub/Ube2N. a Biolayer interferometry table showing the measured on and off rates and the dissociation constants of MavC (C74A) with Ube2N-SS-Ub, Ub, and Ube2N. b Michaelis-Menten parameters of ubiquitination (with respect to Ube2N) and deamidation (with respect to Ub) reactions. c Free Ub binds weakly to MavC. 1H,15N-HSQC NMR spectral overlays of 150 μM 15N-Ub alone (black spectrum) or in the presence (cyan spectrum) of 150 μM C74A-MavC1–384. CSPs are weak or negligible indicating that any interaction between MavC and free Ub is very weak. Addition of 150 μM Ube2N to the sample (red spectrum) has marginal effects on the Ub spectrum, and therefore, does not significantly change Ub binding to MavC at NMR concentrations. d Ube2N-SS-Ub binds to MavC. Spectral overlay of 150 mM Ube2N-SS-2H,15N-Ub alone (black spectrum), or in the presence of 150 μM C74A-MavC1–384 (red spectrum). Only the Ub subunit is isotopically labeled. The Ub subunit was 2H-labeled to allow for NMR observation in high molecular weight complexes. Select Ub subunit resonances are observed to shift or disappear indicating that the Ub subunit interacts with MavC. e A time course comparison of the ubiquitinating activity of MavC on Ube2N-SS-Ub versus free Ube2N and Ub as the substrates. Loading controls for Ube2N-SS-Ub for each time point under non-reducing conditions are also shown. Reactions were subjected to SDS-PAGE and visualized with Coomassie Blue. A control reaction at time 0 is included. Accompanying each time course is a cartoon representation of the reaction components.
are consistent with our solution NMR binding experiments. The surface of the Ube2N subunit formed by Helix1, Loop4, and Loop7 makes extensive contacts with the MavC insertion domain (Fig. 3, Supplementary Fig. 2f). Observable Ub resonances not assigned by inspection and are primarily located in regions of the Ub subunit that do not make contact with MavC (Supplementary Fig. 2i).

Though the Ub subunit does not bind tightly to MavC in solution, the structures reveal a number of potential contacts that we found important for activity. Residues in the MavC HE region (MavC Leu36, Asn39, Glu40, Ile43, and Glu66) interact with the N-terminal β-hairpin turn of Ub as well as the nearby C-terminal Ub tail (Ub Leu8, Thr9, Arg72, and Arg74) (Fig. 4a, b). Around the MavC catalytic triad, numerous contacts are observed, including a rare contact with the di-Pro motif of Ub (Pro37–Pro38) using a CH–O hydrogen bond between the carbonyl group of Ala229MavC and Hδ atoms of the Pro38Ub (ref. 32) (Fig. 4b). Numerous hydrophilic contacts include Asn72MavC positioned to H-bond with the backbone carbonyl of Leu73Ub, Arg121MavC in H-bonding distance to the carbonyl of G35Ub, and the side chains of R126MavC and Thr230MavC poised to interact with the Asp39Ub carboxylate. Mutation of these MavC residues leads to a reduction or complete loss of the ability of MavC to catalyze either transglutamination (using the disulfide conjugate as a substrate) or Ub deamidation (Fig. 4c, d). Mutation of Arg72Ub, one residue that distinguishes Ub from the active site is also shown. MavC active site residues C74, H231, and Q252 are depicted as red sticks.

Fig. 3 Overall structure of Ube2N-SS-Ub bound to MavC. Cartoon representation of the crystal structure of MavC-Ube2N-SS-Ub complex (P65), with MavC depicted in burgundy, Ube2N in green, and Ub in teal. Key domains (CG, core globular domain; HE, helical extension; INS, insertion domain) are labeled. Rotated view depicting a top-down view of the active site is also shown. MavC active site residues C74, H231, and Q252 are depicted as red sticks.

of MavC to recognize this mutant substrate (Supplementary Fig. 3a).

Modeling a Cys side chain in a preferred rotamer orientation in place of MavC Ala74 reveals the γ-S atom poised for nucleophilic attack at Gln40Ub, approaching within 3.0 Å of the carboxamide group (Fig. 4e). Gln40Ub is held in the active site with its side chain C = O group pointing toward the backbone amide groups of the catalytic Cys74MavC and Trp255MavC (H-bonding distance of 2.9 and 3.0 Å, respectively) whereas the NH3 group is in H-bonding contact with the backbone carbonyl group of Thr230MavC (2.8 Å) and the imidazole side chain of His231 (Fig. 4e). The backbone amides would be important for stabilization of the oxyanion transition state and the His231 interaction is likely for proton donation to the leaving ammonia during the thioester step (Fig. 1a). The indole side chain of Trp255MavC is stacked against the Gln40-Gln41 peptide unit of Ub (Fig. 4e), an arrangement that permits the backbone carbonyl of Gln40Ub to come within H-bonding distance from the hydroxyl group of Ser73MavC. Mutation of Trp255 and Ser73 of MavC to alanine results in a significant loss of Ub deamidation and transglutamination activity of the enzyme (Fig. 4c, d). Combined, these interactions appear to fix the Gln40Ub side chain in a reactive arrangement for attack by the nucleophilic Cys to facilitate formation of the thioester intermediate. In this arrangement the NH3 group points toward a solvent-filled area, which would allow the ammonia produced during catalysis to diffuse away from the active site.

In both substrate and product complexes, there are three regions of MavC that interact with Ube2N. Region 1, the MavC insertion domain, makes an overwhelming contribution to Ube2N binding compared to other parts of MavC. Around 500 Å (ref. 2) of the surface area is buried at the interface between MavCNS and Ube2N alone. Accordingly, its deletion results in a dramatic loss of Ube2N binding and no detectable ubiquitination activity (Fig. 5d)21. The MavC insertion domain can be expressed and purified on its own, and a crystal structure of the insertion domain shows that it preserves an identical fold to that in the full MavC protein (Supplementary Fig. 4a). BLI and glutathione-s-transferase (GST) pulldown experiments show that the isolated insertion domain is able to bind to Ube2N independently and with an affinity comparable to full-length MavC (Supplementary Fig. 4b, c). The second Ube2N-interacting region of MavC corresponds to residues in the CG domain, particularly Met317MavC, that interacts with Loop4, the 310-helix containing Lys92Ube2N, and αHelix2 of Ube2N (Fig. 5b). The third region involves contacts between the MavC HE domain and Ube2N αHelix3 (Fig. 5c), an interface largely supported by a network of polar contacts between acidic and basic residues from both MavC and Ube2N. The contacts in this region appear to play a key role as the substrate transitions through the catalytic process. For example, interactions involving Arg63MavC and Lys64MavC with the αHelix3 of Ube2N are observed only in the P65 substrate complex structure, which is closer to the product complex (also in P65) than the other substrate complexes. Consistent with these observations, mutation of Arg63MavC, Lys64MavC, and Glu66MavC in the HE, Phe188MavC, Tyr189MavC, Tyr198MavC, and Glu207MavC in the insertion domain, and Met317MavC in the CG severely impair MavC catalytic activity (Fig. 5d). Tyr47MavC was also chosen as a site for mutation in these mutants because it contributes to hydrophobic interactions at the HE interface. Based on results from the biochemical experiments, we examined the effects of a Tyr47Tyr198/Glu207MavC to alanine triple mutant (the YYE mutant) on MavC-induced Ube2N ubiquitination in cells infected by L. pneumophila. We also created mutants by additionally changing either Met317 or Trp255 to alanine in...
the YYE mutant (YYE/M317A, YYE/W255A). Unlike the single point mutants or YYE, which show appreciable activity under longer reaction times in the transglutaminase assay, these quadruple mutants were much more defective (Supplementary Fig. 3b, c). All of these mutants were translocated into infected cells at levels comparable to that of the wild-type complementation. We then examined the levels of Ube2N ubiquitination in infected cells. We also examined the ability of MavC and the mutants to attenuate NF-κB activation under conditions of TRAF6 overexpression22. Whereas the activity of the YYE mutant has severely impaired the ability to modify Ube2N and displays defects in attenuating NF-κB activation, the quadruple mutants were further impaired to levels comparable to the catalytically inactive C74A mutant, in line with the biochemical activity assay results (Fig. 5e, f, Supplementary Fig. 3c). Collectively these results indicate that the transglutaminase activity of MavC targeting the Ube2N–Ub conjugate is essential for its biological role in attenuating NF-κB response in host cells.

**MavC remodels the Ube2N active site to promote crosslinking.** Remarkably, comparison of substrate and product MavC complexes reveal a progression in the conformation of loops surrounding the Ube2N active site. In one structure containing Ube2N-SS-Ub (Fig. 6a), the E2 conformation is very similar to 49 other structures of Ube2N available in the Protein Data Bank (Supplementary Fig. 4d). The Ube2N active site loop formed by residues 116–123 is the most variable region among Ube2N structures, and in the MavC complex it adopts an altered conformation relative to an average Ube2N structure (Supplementary Fig. 4d). Lys92Ube2N, the residue that will form an isopeptide bond with Gln40Ub, is located in a 310-helix that is common to all other Ube2N structures. Though the complete Lys92Ube2N side chain is not resolved in this structure, its β-carbon is positioned over 16 Å away from the γ-carbon of Gln40Ub. This placement is too far for reaction with a thioester intermediate and isopeptide bond formation, suggesting that a conformational change is required to bring Lys92Ube2N into the MavC active site. The other substrate and product MavC complexes reveal a dramatic change in the conformation of the 310-helix that brings the Ube2N target lysine into position to attack the γ-carbon of Gln40Ub (Fig. 6a–c). Here, Met317MavC, a methionine in MavC, appears to play a critical role in promoting this conformational change. In the early substrate/MavC complex (Fig. 6a), Met317MavC is positioned just below the Ube2N target lysine in an extended conformation. In the attacking substrate/MavC complex, the electron density for the 310-helix is lost suggesting this region adopts multiple conformations in the crystal. Here, Met317MavC has shifted ~4.5 Å relative to the E2 into a hydrophobic pocket of the E2 formed by the movement of the 310-helix. In the product
complex, the 3\textsuperscript{10}-helix is highly extended, with Lys\textsubscript{92}\textsuperscript{Ube2N} positioned in the MavC active site covalently linked to Gln\textsubscript{40}\textsuperscript{Ub} via an isopeptide bond. Notably, the methyl group of Met\textsubscript{317}\textsuperscript{MavC} occupies the position vacated by Ile\textsubscript{90}\textsuperscript{Ube2N} as the 3\textsuperscript{10}-helix unfolds. Consistent with a role in stabilizing the extended Ube2N conformation, mutation of Met\textsubscript{317}\textsuperscript{MavC} abrogates MavC trans-glutaminase activity (Fig. 5d).

Additional interactions may be important for stabilizing the extended structure of Ube2N. In the product complex, the aliphatic portion of the Lys\textsubscript{92} side chain is held in place by van der Waals interactions with Tyr\textsubscript{254}\textsuperscript{MavC} (Fig. 6c, inset) positioning the \(\varepsilon\)-amino group within 4.2 Å from C\(\beta\) of Ala\textsubscript{74}. The proximity of the backbone carbonyl of Thr\textsubscript{230}\textsuperscript{MavC} to Lys\textsubscript{92}\textsuperscript{Ube2N} also indicates a potential hydrogen bond that may stabilize the Lys amino group in a productive orientation. Though the side chain of Lys\textsubscript{92}\textsuperscript{Ube2N} is well-defined in the product complex, the region of Ube2N surrounding this residue still appears to be dynamic. This is inferred from weaker electron density and the relatively high average B-factor of ~70 Å\(^2\) for the residues from 86 to 95, compared to ~40 Å\(^2\) for the whole complex. Thus, it appears that interactions between Ube2N–Ub and MavC lead to large conformational changes in regions surrounding the Ube2N active site, especially unfolding of the Ube2N 3\textsuperscript{10}-helix, which is necessary to position Lys\textsubscript{92}\textsuperscript{Ube2N} into the MavC active site.

**Fig. 5 Binding interface of MavC with Ube2N.** a–c Detailed view of interactions in MavC regions 1, 2, and 3 with Ube2N. Key residues are labeled and represented as stick models. MavC is depicted in burgundy and Ube2N in green. Hydrogen-bonding interactions are given in black and hydrophobic interactions in red. d Comparison of the ubiquitinating activity of wild-type MavC versus mutant proteins using Ube2N-SS-UB as the substrate. Reactions were subjected to SDS-PAGE and visualized with Coomassie Blue. A control reaction without MavC is included. e MavC-mediated ubiquitination of Ube2N during L\textsubscript{p} infection. Cells infected with the indicated L. pneumophila strains were lysed with 0.2% saponin and lysate separated by SDS-PAGE, probed by immunoblotting with antibodies specific for Ube2N (upper panel) and MavC (lower panel), respectively. f The effects of MavC and its mutants on NF-\(\kappa\)B activation. HEK293T cells were transfected with plasmids expressing a luciferase reporter responsive to NF-\(\kappa\)B and Flag-MavC or its mutant. At the same time, a plasmid expressing Renilla luciferase used as an internal control and stimulator TRAF6 were co-transfected. NF-\(\kappa\)B activity was determined by dual luciferase assay. The expression of MavC and its mutants was probed in lysates of transfected cell while tubulin was detected as a loading control. Three independent experiments were done with similar results. Error bars indicate standard error of the mean (SEM).
Conformational dynamics of the MavC insertion domain.

Comparison of the structure of apo-MavC (PDB id 5TSC) to the substrate complexes reveals that Ube2N could not bind to the insertion domain in apo-MavC without steric interference from the MavC HE. However, our NMR titration data show that Ube2N readily binds MavC in solution (Supplementary Fig. 2e, g). This suggests that flexibility of the insertion domain relative to the MavC CG and HE domains are important for function. Indeed, comparison of apo-MavC with substrate-bound complexes reveals that insertion domain undergoes a pronounced 30° rigid-body rotation (Fig. 6d, e) that would enable MavC to accommodate the Ube2N-SS-Ub substrate. As the reaction proceeds further, the insertion domain undergoes a second rigid-body pendulum movement that helps to bring Ube2N near active site.

MavC can target the Uev1a/Ube2N–Ub conjugate. Essential for Ube2N-dependent activation of NF-κB is the synthesis of K63-linked poly-Ub chains. This requires complex formation of

Fig. 6 Conformational dynamics of MavC and remodeling of the Ube2N active site. Superposition of Ube2N in three different structures (a–c) reveal the remodeling of Ube2N loops that occurs upon interaction with MavC. A comparison of crystal structures of MavC bound to Ube2N-SS-Ub in the P65 space group (a), in the R3 space group (b), and MavC bound to the product Ub–Ube2N (c) captures the progression of the 310-helix of Ube2N from canonically structured to destabilized to unfolded. The β-carbon of the target Lys92Ube2N is highlighted with a blue sphere. Note the changing position of MavC M317 that accompanies unfolding of the Ube2N 310 helix. Inset e shows interactions involving Lys92Ube2N in the active site of MavC as observed in the product-bound structure. d Structural alignment of apo-MavC (cyan) with substrate-bound MavC (R3, burgundy). Insertion domain is highlighted with a box. e Zoomed view of the insertion domain of apo and bound MavC (color coded as previously), depicting the rigid-body rotation upon Ube2N-SS-Ub binding. f Structural alignment of the two substrate-bound crystal structures of MavC (R3: MavC in burgundy, Ube2N in green. P65: MavC in yellow, Ube2N in magenta). Insertion domain is highlighted with a box. g Zoomed view of insertion domain in both substrate-bound crystal forms of MavC depicting the pendulum rotation required to bring Ube2N near active site.
Ube2N with an E2 variant protein, such as Uev1a, that binds the acceptor Ub and directs synthesis of K63 linkages. Thus, it is likely that MavC targets Uev1a/Ube2N–Ub conjugates in order to disrupt NF-κB activation. Uev1a binds tightly to Ube2N33, interacting with residues in β-strands 3 and 4 of Ube2N and some of the interconnecting loops, including the extended loop that leads into the E2 active site. Our structures predict that this interaction site is solvent accessible in the MavC complex, and that MavC should also be able to target the Uev1a/Ube2N–Ub conjugate. Accordingly, we demonstrated that Uev1A can bind to Ube2N-SS-Ub as deduced from co-elution of the two proteins during size-exclusion chromatography (Supplementary Fig. 5a). This prediction is also borne out by in vitro activity data that shows increasing additions of Uev1A to the reaction mixture does not inhibit the ability of MavC to catalyze the intramolecular transglutamination of Ube2N and Ub (Supplementary Fig. 5b). In agreement with these results, our structural analysis reveals that Uev1A binding to Ube2N–Ub is unlikely to interfere with MavC binding (Supplementary Fig. 5c).

### Discussion

MavC catalysis presents a remarkable example of a ubiquitination reaction achieved through transglutaminase chemistry that does not require nucleotide-dependent activation of Ub. The result is isopeptide crosslinking of Ub to a specific target, Ube2N. Though other E2s harbor a structurally equivalent target lysine residue, selectivity for Ube2N is achieved by binding the same interface recognized by cognate eukaryotic E3 ligases (Supplementary Fig. 3b–d). Furthermore, low concentrations of MavC (nM) effectively target and inhibit both activated Ube2N–Ub conjugate and the Uev1a/Ube2N–Ub complex by catalyzing the synthesis of an intramolecular isopeptide bond adjacent to the E2 active site. Catalysis requires extensive remodeling of loops surrounding the E2 active site, structural changes that have not been previously observed in other Ube2N structures. Targeting a specific E2–Ub conjugate allows *L. pneumophila* to modulate specific host cellular processes instead of a systemic effect on the entire host Ub landscape.

With an approximate cellular concentration of 2 µM, Ube2N is among the most abundant E2 enzymes in cells, existing mostly as the thioester-linked Ube2N among the most abundant E2 enzymes in cells, existing mostly as processes instead of a systemic effect on the entire host Ub conjugate allows observed in other Ube2N structures. Targeting a specific E2–Ub conjugate...
and NEDD8 deamidation through a lower Ub affinity. Instead, it preferentially attacks the Ube2N–Ub conjugate to turn off Ube2N’s ability to generate K63 poly-Ub chains. Despite both free Ub/NEDD8 deamidation and Ube2N ubiquitination ultimately being shown to lead to decrease in NF-κB activation, we speculate that the divergence of MavC occurred to accommodate other L. pneumophila effectors that utilize the host’s free Ub such as the E3 ligases LegU1 and SidC36,37 and the noncanonical ligases of the SidE family. Poisoning of the host cell’s supply of Ub by deamidation could antagonize these other effectors’ activity. Therefore, MavC may satisfy a need for an alternative method of attenuating host immune signaling without perturbing the free Ub pool. How hampering the host’s ability to make K63 poly-Ub chains may lead to other cellular effects remains to be elucidated.

Methods
Cloning, expression, and purification of recombinant proteins. MavC1–344 was PCR amplified from a plasmid encoding full-length MavC or MavC C74A using a PCR premix kit (Bioneer) cloned into pGEX-6p-1 plasmid (GE Healthcare). The resulting expression plasmid was transformed into BL21 (DE3) strain of E. coli (Novagen). Cells were grown in LB media at 37 °C to an OD600 of 0.6, cooled to 18 °C, and induced overnight at the reduced temperature by addition of 0.35 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG). The protein eluted off the column was dialyzed against two changes (4 L) of dialysis buffer (250 mM Tris pH 8, 500 mM KCl, and 10 mM reduced glutathione). The protein eluted from the column was dialyzed against two changes (4 L) of dialysis buffer (1× PBS supplemented with 0.5 M KCl and 1 mM DTT) at 4 °C to remove excess glutathione while being incubated with GST-tagged PreScission Protease as per the manufacturer’s recommendation (GE Healthcare). The dialyzed sample was further passed through 5 mL of pre-equilibrated GST column to capture all of the MavC mutants used in this study, E1, Ubc13, Ube2S, and Uev1A were also expressed and purified similarly. E1, Ubc13, Ube2R1, and Ube2S constructs were obtained from Genentech, and Uev1A was obtained from Yusuke Sato (University of Tokyo).

Ube2N (full-length) cloned into pET-SUMO was purchased from Addgene (Plasmid #51131). The plasmid, transformed into BL21 (DE3) strain of E. coli, was expressed, lysed, and centrifuged same as the MavC constructs. The supernatant was passed through a pre-packed 5 mL HisTrap column (GE Healthcare) pre-equilibrated with binding buffer. Once the supernatant was loaded the resin was washed with five column volumes of binding buffer to wash off any unbound protein. This was followed by a 50 mM imidazole wash (binding buffer supplemented with 50 mM imidazole). The bound fusion protein was eluted with

![Fig. 7 Overall scheme of MavC-catalyzed ubiquitination](image-url)
elution buffer (1× PBS supplemented with 300 mM imidazole). The protein eluted
NATURE COMMUNICATIONS | (2020)11:2365 | https://doi.org/10.1038/s41467-020-16211-x | www.nature.com/naturecommunications
of protein samples from every stage of expression and purification were monitored by
analysis, were pooled, concentrated, and exchanged into protein storage buffer (50 mM
precipitates, if necessary. The cleared supernatant was loaded onto a self-packed
crystallized at 37 °C for 3 h in 50 mM Tris pH 7.4, 100 mM NaCl and 1 mM DTT, then
approximately 10 mg/mL. Crystals were grown by hanging drop vapor diffusion method at
the disulfide-linked protein complex, Ube2N-SS-Ub, was isolated by size-exclusion chromatography (Superdex
the initial search model used was the insertion domain from MavC7
−[\text{Gly}]_76\text{Ub}
NATURE COMMUNICATIONS | https://doi.org/10.1038/s41467-020-16211-x
NATURE COMMUNICATIONS | (2020)11:2365 | https://doi.org/10.1038/s41467-020-16211-x | www.nature.com/naturecommunications
harvested and kept for protein crystallization.

\textbf{Structure determination and refinement.} The structures of MavC_{1-284}–Ube2N-SS-Ub complex in three different space groups were determined by maximum likelihood molecular replacement using the program PHASER40 from CCP4 suite31. The initial search models used were native MavC_{1-284} (PDB code 5TSC31), Ube2N (PDB code 2C2V42), and Ub (PDB code 1UBQ43). The asymmetric unit consists of one complex in each of the three space groups. Iterative rounds of model building with the program COOT34 and refinement with the program PHENIX45 was used to arrive at the final structures that were validated through MolProbity36 and deposited with the Protein Data Bank (PDB code 6U5B). The structure of the complex between MavC_{1-284} and Ube2N-SS-Ub in the P63 space group (attacking complex I), determined at 1.9 Å resolution (Supplementary Table 2), has one molecule of MavC_{1-284} engaging with one molecule of Ube2N-SS-Ub in the asymmetric unit. Analysis of the Ramachandran plot37 indicated that 98.8% of residues fall in the most favored region and 1.2% in the additionally allowed regions of the plot, none in the disallowed region of the plot. Electron density for almost all of the residues from MavC, Ub, and Ube2N are well resolved. Residues from 89 TO 94 in Ube2N have almost no density and hence have not been modeled in the structure. A total of 298 water molecules (an average B-factor of 42.6 Å²) were observed in the asymmetric unit (PDB code for the coordinate and the reflection file is 6U5B).

The structure of the complex between MavC_{1-284} and Ube2N-SS-Ub in the C222 space group (attacking complex II), determined at 2.34 Å resolution (Supplementary Table 2), has one molecule of MavC_{1-284} engaging with one molecule of Ube2N-SS-Ub in the asymmetric unit. Analysis of the Ramachandran plot37 indicated that 97.8% of residues fall in the most favored region and 2.2% in the additionally allowed regions of the plot, none in the disallowed region of the plot. Electron density for almost all of the residues from MavC, Ub, and Ube2N are well resolved. Residues from 89 to 94 in Ube2N have almost no density in this complex as well and hence have not been modeled in the structure. A total of 168 water molecules (an average B-factor of 50.7 Å²) were observed in the asymmetric unit (PDB code for the coordinate and the reflection file is 6UMS).

The structure of the complex between MavC_{1-284}–Ube2N complex (product complex) was determined by maximum likelihood molecular replacement using the program PHASER40 from CCP4 suite31. The initial search models used were native MavC_{1-284} (PDB code 5TSC31), Ube2N (PDB code 2C2V42), and Ub (PDB code 1UBQ43). The asymmetric unit consists of one complex. Iterative rounds of model building with the program COOT34 and refinement with the program PHENIX45 resulted in the final structure for all data between 40 and 2.1 Å resolution (Supplementary Table 2). The final structure was validated through MolProbity36 and deposited in the Protein Data Bank (PDB code 6P5B). The structure of the complex between MavC_{1-284} and ubiquitinated Ub (Ub–Ube2N), determined at 2.07 Å resolution (Supplementary Table 2), has one molecule of MavC_{1-284} engaging with one molecule of Ub–Ube2N in the asymmetric unit. Analysis of the Ramachandran plot37 indicated that 97.9% of residues fall in the most favored region and 2.1% in the additionally allowed regions of the plot, none in the disallowed region of the plot. Electron density for the main chain of the MavC_{1-284} construct along with four non-native residues at the N-terminal end remaining after the cleavage of the GST affinity tag are well resolved in the crystal structure. A total of 197 water molecules (an average B-factor of 43.4 Å²) were observed in the asymmetric unit. A separate model with the catalytic Cys of MavC included was generated using COOT.

The MavC insertion domain (MavC_{285-384}) structure was determined by maximum likelihood molecular replacement using the program PHASER40 in the PHENIX35 suite. The initial search model used was the insertion domain from MavC_{285-384} (PDB code 5TSC31), and the asymmetric unit contained two molecules, which were consistent with the formation of a crystallographic dimer. The structure was taken through multiple steps of model building with the program COOT34 and
refinement with the program PHENIX which resulted in a final structure for all data between 29.8 and 1.53 Å resolution (Supplemental Table 2). The final structure was validated through MolProbity and deposited with the Protein Data Bank (PDB code for the coordinates and structure factors is 6SPU).

Residues where poor electron density was observed were modeled as either alanines or glycines. Interface area and residues at the interface in all the complexes presented in this study were computed using the web-based server, PISA, at the European Bioinformatics Institute [http://www.ebi.ac.uk/pdb/prot_int/pistart.html]. All structures in this work were rendered and presented using PyMol (http://pymol.org).

Mutagenesis. Plasmids harboring the desired mutations and/or truncations of MavC were constructed via site-directed mutagenesis using mutagenic primer pairs. The resulting mutant PCR products were digested by DpnI to remove the methylated templates and transformed into E. coli DH5α (home-made competent cells). Presence of desired mutations was confirmed by sequencing before being transformed into E. coli BL21 (DE3) for protein expression and subsequent purification, similar to the aforementioned MavC constructs.

Primers used for mutagenesis in this study are listed in Supplementary Table 1.

GST pulldown assays. The pulldown assays were performed with GST-fusion proteins of MavCΔ462, MavCΔNS, and MavCΔINS as probe proteins to pulldown Ube2N. A 100 µL of a 50% slurry of glutathione-agarose beads were equilibrated with 10× bed volume of 1× PBS buffer; pH 7.4. The beads were then centrifuged for 5 min at 5000 rpm and the supernatant discarded. This wash step was repeated twice more, each time with a 100 µL of a 1× PBS buffer solution. The probe protein was used to charge the beads. These were then incubated at 4°C with end-over-end mixing for 4 h, to ensure that the bait protein bound to the beads. The loaded beads were centrifuged for 5 min at 5000 rpm and the flow through collected. The beads were washed as before and the wash collected after each step. Following the washes, the charged beads were incubated with 100 µL of a 100 µM stock of Ube2N. Binding was allowed to proceed overnight at 4°C with end-over-end mixing. After incubation, the beads were centrifuged and washed as above, collecting the flow through and wash at each step. Proteins were eluted by incubating the beads in 50 µL of GST elution buffer (250 mM Tris pH 8, 500 mM KCl, and 10 mM reduced glutathione) for 15 min at room temperature followed by centrifugation. The elution step was repeated once more. The collected samples were analyzed by SDS-PAGE.

Biolayer interferometry. Inactive (C74A) poly-His-tagged MavC constructs for use in the BLI studies were generated using two-step PCR (Megaprimer method) and verified using DNA sequencing. Primers used for cloning the constructs in this study are listed in Supplementary Table 1. The His-tagged proteins were expressed and purified as described above for Ube2N. Homogeneity of all the purified proteins was confirmed by SDS-PAGE analysis. The MavC constructs used for these studies were diluted in BLI buffer (1× PBS containing 0.05% v/v Tween-20 and 0.1% w/v BSA) to a concentration of 25 µg/mL. The analytes were also diluted in the same buffer at the following concentrations: Ub (2 µM), Ube2N (200 µM), Ube2N-SS-Ub (100 µM), and Ub-Ube2N (100 µM). Serial dilutions of each analyte were prepared, in replicates of three, for analysis. One Ni-NTA biosensor was used for each Kq measurement, dipping the MavC protein loaded tip into wells that contained an analyte, starting with the lowest concentration of analyte first. The direct binding experiment was performed for 120 s for association and 180 s for dissociation in BLI buffer using a bilayer interferometer (BLI), Octet Red 96 system ( Pall ForteBio, Corp., Menlo Park, CA, USA), and data acquired using the ForteBio Data Acquisition 8.2 software (Pall ForteBio Corp., Menlo Park, CA, USA). Association responses (from 110 to 115 s) were averaged and plotted in BAL Octet Data Analysis Software. The data were fit to a non-linear regression one site—specific binding model to determine the Kq.

Ubiquitination and deamidation assays. To analyze the ubiquitinating activity of MavC mutants, purified MavCΔ462 constructs (wild type or mutants) were co- incubated with 0.1 µM Ube2N-SS-Ub, and incubated at 37°C for 30 min in reaction buffer (50 mM Tris pH 7.4, 100 mM NaCl). The reaction products were analyzed by SDS-PAGE and visualized with Coomassie Blue. To analyze the quadruple mutants an compare them with other mutants, an increased incubation time of 60 min and a [MavC] of 0.5 µM was utilized. On the other hand, to analyze the ubiquitinating activity of MavCΔINS and also the ubiquitinating activity of MavC against Ubc13, UbcE2S, and Ube2E1R, 0.5 µM MavC, 25 µM Ube2N, and an extended incubation time of 60 min was used, and the Ub concentration was increased to 100 µM in an attempt to promote ubiquitination.

Ub deamidating assays were performed by combining purified MavCΔ462 constructs (wild type or mutants) with Ub at a final concentration of 0.5 µM enzyme and 100 µM Ub. The reactions were incubated at 37°C for 30 min in reaction buffer (50 mM Tris pH 7.4, 100 mM NaCl). The reaction products were analyzed by Native-PAGE and visualized with Coomassie Blue.

MavC’s deamidating activity on the disulfide conjugate was tested by combining purified MavCΔ462 with Ube2N-SS-Ub at a final concentration of 0.005 µM MavC and 25 µM Ube2N-SS-UB and incubated at 37°C for 30 min in reaction buffer (50 mM Tris pH 7.4, 100 mM NaCl). The reaction products were analyzed by Native-PAGE and visualized with Coomassie Blue. As a control to observe the migration of deamidated Ube2N-SS-Ub, a reaction was run utilizing the known deamidase Cif at a concentration of 0.5 µM.

To determine the Michaelis–Menten kinetic parameters of the Ub deamidation activity of MavC, reactions were conducted with MavC (0.5 µM), and varying concentrations of Ube2N at 37°C for 30 min. Reactions were analyzed with Firefly luciferase substrate and measured Firefly luciferase activity. To compare the ability of E1 to charge Ube2N versus Ube2N, a reaction mixture of 0.5 µM E1 enzyme, 200 µM Ube2N or Ube2N-SS-Ub was incubated in a reaction buffer of 50 mM Tris pH 7.4, 100 mM NaCl, 2.5 mM ATP, 5 mM MgCl2. Reactions were allowed to proceed for 30 min at 37°C before quenching with either reducing or non-reducing SDS-PAGE buffer, separated by SDS-PAGE, and visualized with Coomassie Blue.

MavC-mediated ubiquitination of Ube2N during L. pneumophila infection. L. pneumophila strains were grown to post-exponential phase (OD562 = 3.2–3.8) in A5 medium at 37°C and then induced for 4 h with LPS and defagulated. L. pneumophila strains were obtained from prior studies,22,25,26,28 LPS was isolated from cells or U937 cells were infected with L. pneumophila strains at an MOI of 10 for 2 h. Cells were washed three times with PBS and then lysed with 0.2% saponin on ice for 30 min. Cell lysates were resolved by SDS-PAGE and probed with MavC (1:5000 dilution)22–specific and Ube2N (1:1000 dilution)—specific antibodies. Anti-Ube2N antibody was purchased from Thermo Fisher Scientific (catalog # 37-1100). Tubulin and ICDH were used as a loading controls and probed using anti-tubulin antibody (1:10,000 dilution) from DSHB (catalog # E7) and anti-ICDH (1:10,000)22.

E1 charging assay. To compare the ability of E1 to charge Ube2N versus Ube2N, a reaction mixture of 0.5 µM E1 enzyme, 200 µM Ube2N or Ube2N-SS-Ub was incubated for 60 min in a reaction buffer of 50 mM Tris pH 7.4, 100 mM NaCl, 2.5 mM ATP, 5 mM MgCl2. Reactions were allowed to proceed for 30 min at 37°C before quenching with either reducing or non-reducing SDS-PAGE buffer, separated by SDS-PAGE and visualized with Coomassie Blue.

NF-κB activation assay. HEK293T cells were grown to 70% confluence in 24-well plates, and transfected with 100 ng NF-κB reporter plasmids and 10 ng of plasmid encoding expression of GFP. Six hours after transfection cells were transfected with 96-well plate. Depending on the experimental control. Four hundred nanograms 4xFlag-MavC vector or its mutant and 400 ng 4xFlag-Traf6 were co-transfected at the same time. After 24 h, the cells were then collected and lysed for NF-κB luciferase reporter assay following the manufacturer’s protocols (Promega cat. no. E1910). Briefly, cells were lysed with 100 µL RIPA buffer and transferred to 1× Binding buffer (Promega) as internal control. Luciferase assay buffer containing Firefly luciferase substrate and measured Firefly luciferase activity. After that, 100 µL Luciferase Stop and Glu reagent was added.
and Renilla luciferase activity measured. The expression of MavC or its mutant was
probed in lysates of transfected cells and the bands shown are representatives of at
least three independent experiments. Anti-MavC antibody22 was used at a dilution
of 1:5000. Tubulin was used as a loading control and probed using anti-tubulin
antibody (1:10,000 dilution) from DSHB (catalog # E7).

Statistical methods. The gels presented in this study are representative of three
different experiments performed independently, with similar results obtained
(Figs. 2a, 4c, d, e, Supplementary Figs. 1a, 2a, b, 3a, c, 3b, 4b, c, and 5a, b). No statistical
methods was used to predict sample size. The experiments were not randomized
and were not performed with blinding to the conditions of the experiments.

Reporting summary. Further information on research design is available in
the Nature Research Reporting Summary linked to this article.

Data availability
Coordinates of all five structures have been deposited into the Protein Data Bank
under accession codes 6LMD, 6LJH, 6UMS, 6F5B and 6F5H. The source data underlying
Figs. 2a, b, c, d, e, f, and Supplementary Figs. 1a, 2a, b, 3a, c, 3b, 4b, c, and 5a, b are
provided as a Source Data file. Other data are available from the corresponding authors
upon reasonable request.

Received: 24 October 2019; Accepted: 14 April 2020;
Published online: 12 May 2020

References
1. Konderman, D. & R ape, M. The ubiquitin code. Annu. Rev. Biochem.
https://doi.org/10.1146/annurev-biochem-060310-170328 (2012).
2. Hershko, A. et al. Components of ubiquitin-protein ligase system. J. Biol.
Chem. 258, 8206–8214 (1983).
3. Ebner, P., Versteeg, G. A. & Reda, F. Ubiquitin enzymes in the regulation of
immune responses. Crit. Rev. Biochem. Mol. Biol. https://doi.org/10.1080/10409238.2017.1325829 (2017).
4. Grabbe, C., Hunsják, K. & Dikic, I. The spatial and temporal organization
of ubiquitin networks. Nat. Rev. Mol. Cell Biol. https://doi.org/10.1038/nrm3099 (2011).
5. Bhavsar, A. P., Gutmann, J. A. & Finlay, B. B. Manipulation of host-cell
pathways by bacterial pathogens. Nature https://doi.org/10.1038/nature06247 (2007).
6. Angot, A., Vergunst, A., Genin, S. & Peeters, N. Exploitation of eukaryotic
pathways by bacterial pathogens. Trends Biochem. Sci. https://doi.org/10.1016/j.
tibs.2018.11.006 (2019).
7. Luo, Z.-Q. & Isberg, R. R. Multiple substrates of the Legionella pneumophila
Dot/Icm system identified by interbacterial protein transfer. Proc. Natl.
Acad. Sci. USA https://doi.org/10.1073/pnas.0309161101 (2004).
8. Burstein, D. et al. Genomic analysis of 38 Legionella species identifies large
and diverse effector repertoires. Nat. Genet. 48, 167–175 (2016).
9. Qu, J. & Luo, Z.-Q. Legionella and Coxella effectors: strength in diversity and
activity. Nat. Rev. Microbiol. 15, 591–605 (2017).
10. Poulignier, A., Vieusseux, D. et al. Discovery of ubiquitin deamidases in the pathogenic
arsenal of Legionella pneumophila. Cell Rep. 23, 568–583 (2018).
11. Gan, N., Nakayasu, E. S., Hollenbeck, P. J. & Luo, Z.-Q. Legionella
pneumophila inhibits immune signalling via MavC-mediated
transglutaminase-induced ubiquitination of UBE2N. Nat. Microbiol. 4, 134 (2019).
12. Song, L. & Luo, Z.-Q. Post-translational regulation of ubiquitin signaling.
13. Cui, J. et al. Glutamine deamidation and dysfunction of ubiquitin/NEDD8
ubiquitin networks. Cell Biol. https://doi.org/10.1038/ca.2016.35 (2016).
14. Eddins, M. J., Carlile, C. M., Gomez, K. M., Pickart, C. M. & Wolberger, C.
Conjugating enzyme using a novel covalent intermediate. J. Biol.
Chem. https://doi.org/10.1038/mc.200520500 (2005).
15. Pruneda, J. N., Stoll, K. E., Bolton, L. J., Brzovic, P. S. & Klevert, R. E. Ubiquitin in
motion: structural studies of the ubiquitin-conjugating enzyme–ubiquitin
conjugate. Biochemistry 50, 1642–1633 (2011).
16. Bhattacharyya, R. & Chakrabarti, P. Stereospecific interactions of proline
residues in protein structures and complexes. J. Mol. Biol. 331, 925–940
(2003).
17. VanDenDekker, A. D., Hofmann, R. M., Tsui, C., Pickart, C. M. & Wolberger, C.
Molecular insights into polyubiquitin chain assembly: crystal structure of the
Mms2/Ubc13 heterodimer. Cell 105, 711–720 (2001).
18. Chen, V. B. et al. MolProbity: all-atom structure validation for
protein crystallography. Acta Crystallogr. D Biol. Crystallogr. 66, 486–501 (2010).
19. Adams, P. D. et al. PHENIX: a comprehensive python-based system for
macromolecular structure solution. Acta Crystallogr. D Biol. Crystallogr. 66, 213–221 (2010).
20. Luan, V. R. et al. MolProbity: all-atom structure validation for
macromolecular crystallography. Acta Crystallogr. D Biol. Crystallogr. 66, 12–21 (2010).
21. Valleau, D. et al. Discovery of ubiquitin deamidases in the pathogenic
arsenal of Legionella pneumophila. Cell Rep. 23, 568–583 (2018).
22. Fu, P. et al. Complex structure of Ospl and Ubcl3: the molecular basis of
Ubc13 deamidation and convergence of bacterial and host E2 recognition.
PLoS Pathog. https://doi.org/10.1371/journal.
ppt.0033003 (2007).
23. Hubeight, I. & Rohde, J. R. Hell’s BELs: bacterial E3 ligases that exploit the
eukaryotic ubiquitin signaling pathways by effectors translocated by bacterial type III
and type IV secretion systems. PLoS Pathog https://doi.org/10.1371/journal.
ppt.1004255 (2014).
24. Pruneda, J. N. et al. The molecular basis for ubiquitin and ubiquitin-like
specificities in bacterial effector proteases. Mol. Cell 63, 261–276 (2016).
25. Sheedlo, M. J. et al. Structural basis of substrate recognition by a bacterial
deuibiquitinase important for dynamics of phagosome ubiquitination. Proc.
Natl. Acad. Sci. USA 112, 15090–15095 (2015).
26. Sanada, T. et al. The Shigella flexneri effector Ospl deamidates UBC13 to
dampen the inflammatory response. Nature https://doi.org/10.1038/
nature10894 (2012).
27. Fu, P. et al. Complex structure of Ospl and Ubc13: the molecular basis of
Ubc13 deamidation and convergence of bacterial and host E2 recognition.
PLoS Pathog. https://doi.org/10.1371/journal.ppat.1003322 (2013).
28. Song, L. & Luo, Z.-Q. Post-translational regulation of ubiquitin signaling.
J. Biol. Chem. 218, 1776–1786 (2019).
29. Cui, J. et al. Glutamine deamidation and dysfunction of ubiquitin/NEDD8
induced by a bacterial effector family. Science https://doi.org/10.1126/
science.1193844 (2010).
30. Bhogaraju, S. et al. Phosphoribosylation of ubiquitin promotes serine
ubiquitination and impairs conventional ubiquitination. Cell 167, 1636–1649.
e13 (2016).
31. Qu, J. et al. Ubiquitination independent of E1 and E2 enzymes by bacterial
effectors. Nature 533, 120–124 (2016).
32. Kotewicz, K. M. et al. A single Legionella effector catalyzes a multistep
ubiquitination pathway to rearrange tubular endoplasmic reticulum for
replication. Cell Host Microbe 21, 169–181 (2017).
33. Puvar, K., Luo, Q. Z. & Das, C. Uncovering the structural basis of a new twist
in protein ubiquitination. Trends Biochem. Sci. https://doi.org/10.1016/j.
tibs.2018.11.006 (2019).
Acknowledgements
This work was funded by National Institute of Health Grants R01GM126296 (to C.D.), T32GM132024 (to S.K.), R01AI127465 (to Z-Q.L.), and R01 GM088055 (to R.E.K.). We thank our staff contacts, Monica Green and Joe Brunzelle, on the LS-CAT beamlines, 21-ID-F and 21-ID-G, at the Advanced Photon Source (Argonne National Laboratory) for their support during X-ray data collection. We thank Ronald Stenkamp for help comparing Ube2N structures. We acknowledge Genentech, Inc. for the gifts of the E1, Ubiquitin, Ube2R1, and Ube2S plasmids, and Yusuke Sato (University of Tokyo) for the Uev1A plasmid. The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of NIH.

Author contributions
K.P., S.I., Z-Q.L., P.S.B., R.E.K., and C.D. designed and interpreted experiments. P.S.B. and R.E.K. planned and carried out NMR analyses and designed biochemical assays. J.F. and Z-Q.L. performed Legionella infection and MavC transfection experiments. S.K. performed BLI analyses. K.L.N.T. performed pulldown experiments. K.P and S.I. performed all other experiments including crystallization and structure determination of the protein complexes. K.P., S.I., P.S.B., R.E.K., and C.D. wrote the paper with editorial input from all authors.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41467-020-16211-x.

Correspondence and requests for materials should be addressed to R.E.K. or C.D.

Peer review information Nature Communications thanks Yongqun Zhu and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2020