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The *Legionella* effector WipB is a translocated Ser/Thr phosphatase that targets the host lysosomal nutrient sensing machinery

Marie S. Prevost, Nikos Pinotsis, Maud Dumoux, Richard D. Hayward & Gabriel Waksman

*Legionella pneumophila* infects human alveolar macrophages and is responsible for Legionnaire’s disease, a severe form of pneumonia. *L. pneumophila* encodes more than 300 putative effectors, which are translocated into the host cell via the Dot/Icm type IV secretion system. These effectors highjack the host’s cellular processes to allow bacterial intracellular growth and replication. Here we adopted a multidisciplinary approach to investigate WipB, a Dot/Icm effector of unknown function. The crystal structure of the N-terminal domain at 1.7 Å resolution comprising residues 25 to 344 revealed that WipB harbours a Ser/Thr phosphatase domain related to the eukaryotic phospho-protein phosphatase (PPP) family. The C-terminal domain (residues 365–524) is sufficient to pilot the effector to acidified LAMP1-positive lysosomal compartments, where WipB interacts with the v-ATPase and the associated LAMTOR1 phosphoprotein, key components of the lysosomal nutrient sensing (LYNUS) apparatus that controls the mammalian target of rapamycin (mTORC1) kinase complex at the lysosomal surface. We propose that WipB is a lysosome-targeted phosphatase that modulates cellular nutrient sensing and the control of energy metabolism during *Legionella* infection.
(PPP) family, and a C-terminal domain sufficient to pilot WipB to the acidified LAMP1-positive lysosomal compartments in eukaryotic cells, where WipB interacts with the v-ATPase and the associated LAMTOR1 phosphoprotein, key components of the lysosomal nutrient sensing (LYNUS) apparatus.

Results and Discussion
The structure of the WipB N-terminal domain reveals a phosphatase domain. WipB was purified following over-expression in E. coli as described in Materials and Methods. However, we observed rapid breakdown of the protein into shorter fragments (Fig. 1a), the shortest of which was identified by mass spectrometry to contain the first 364 residues of the protein. A construct encoding this apparently stable fragment was generated and the protein expressed and purified (Fig. 1a). This fragment, WipB1-364, was stable but did not crystallise. Thus, the construct was further modified to exclude the regions of the N- or C-terminal sequence predicted to have no secondary structures (regions 1–24 and 345–364). The resulting fragment, WipB25-344, also yielded a stable protein, which in contrast to WipB 1-364 crystallised readily. The crystals diffracted to a resolution of 1.7 Å (Table 1). The structure was solved using the Molecular Replacement method, with the recently solved structure of WipA (a protein with 34% identity at amino acid sequence level (Fig. S1)) serving as a search model12. Attempts to similarly produce the C-terminal domain of WipB failed, as this region of the protein appears to be unstable when expressed in bacteria. Thus, WipB appears to contain two domains, a structured N-terminal domain and a possibly less stable C-terminal domain.

WipB25-344 crystallizes as a dimer in the asymmetric unit. While the two chains superpose very well (root-mean-square deviation (RMSD) in Cα atoms of 0.5 Å), chain A exhibits overall lower B-factors (Fig. S2a). In chain A, density for residues 332–344 is missing while the same region is resolved in chain B (Fig. S2b). This region in chain B interacts with chain A, probably an artefact of crystal packing as WipB25-344 is monomeric in solution.
WipB 25-344 forms a compact structure consisting of a central β-sandwich and 11 α-helices, 4 α-helices on each side of the β-sandwich (in cyan (α1-4) and red (α8-11), respectively, in Fig. 1b,c) with three more α-helices (α5-7) capping the structure (in green in Fig. 1b,c). The structure can be further subdivided in an N-terminal lobe consisting of α1-4 and β1-3 (cyan and blue in Fig. 1b,c) on the one hand and a C-terminal lobe consisting of α8-11 and β4-6 (in red and yellow in Fig. 1b,c) on the other hand. The structure of WipB 25-344 is very similar to that of WipA 24-435 determined previously (the structures align with a root-mean-square deviation in Cα atoms of 1.9 Å over 279 WipB residues; Fig. S2c). However, WipA differs from WipB in including a very large α-helical hairpin (Fig. S2c).

A search for similar structures using the DALI server highlighted several hydrolases among the highly scored homologous structures, including the catalytic domain of a number of serine/threonine (Ser/Thr) protein phosphatases including PP1, PP2A, PP2B, and PP5, important members of the larger phospho-protein phosphatase (PPP) family of proteins. Structural superposition of WipB 25-344 with these eukaryotic phosphatases (see Fig. S1 for sequence alignment with a number of PPPs, and Figs S2d and 2a for structural superposition with PP2B) reveals a common structural core aligning with an RMSD in Cα atoms of 2 Å. This core consists of the central β-sandwich together with the flanking helices α1, α4, and α6 on one side and α8 and α11 on the other. Importantly, these regions harbour signature motifs conserved in PPPs, notably Ser/Thr phosphatases (Fig. 2a and S1), and these motifs cluster in one particular region of WipB 25-344 on the loops connecting α1 and α1 (GDLHA), 32 and α4 (GDELVDR), and 33 and α5 (SNG). In this region, the side chains of Asp 32 and His 34 (in GDLHA), Asp 118 and Asp 122 (in GDELVDR) and of His 151 (in NHG) form a site which in PP2B and other Ser/Thr phosphatase has been shown to be the catalytic site of these enzymes (Fig. 2b). Two additional residues are located in this cluster, His 231 and Asp 331, both conserved and known to be important for activity in PP2B and other PPPs (Fig. 2b).

**WipB is a Ser/Thr phosphatase.** The structural homology of WipB 25-344 with phosphatases of the PPP family suggests that WipB might contain a Ser/Thr phosphatase activity. To test this hypothesis, we investigated

| Data Collection | WipB 25-344 |
|-----------------|-------------|
| Beamline        | PX13 (EMBL-PETRA III) |
| Wavelength (Å)  | 0.99999 |
| Resolution Range (Å) | 48.83–1.70 (1.74–1.70) |
| Space group     | P2₁ |
| Cell parameters a, b, c, β (Å, grad) | 49.40, 79.10, 49.70, 98.70 |
| Total reflections | 240,903 (16,864) |
| Unique reflections | 66,193 (4,732) |
| Multiplicity    | 3.6 (3.6) |
| Completeness (%) | 99.3 (97.1) |
| Mean I/σ(I)     | 8.73 (1.21) |
| Wilson B-factor (Å²) | 24.67 |
| R(sin) (%)      | 11.6 (115.1) |
| CC1/2           | 0.996 (0.480) |

**Refinement**

| R(sin)/Rfree (%) | 18.7/21.7 |
| CC(work)/CCfree | 0.955/0.938 |
| Protein atoms   | 5089 |
| Solvent molecules | 484 |
| B-factor (Å²)   | 22.10 |
| Protein         | 60.91 |
| Solvent         | 56.39 |

**Ramachandran Plot**

| Favor (%)  | 96.15 |
| Allowed (%) | 3.69 |
| Outliers (%) | 0.16 |
| Clash score | 5.34 |

**Rmsd**

| Bonds (Å) | 0.007 |
| Angles (grad) | 0.880 |
| PDB code | 5NNY |

Table 1. Data collection and refinement statistics. Information for the highest resolution shell is given in parentheses.
the ability of the WipB N-terminal domain (residues 1-364) to catalyse dephosphorylation reactions in vitro using a model phosphothreonine peptide with sequence RRA(pT)V A, a peptide derived from the rat liver pyruvate kinase and routinely used to monitor Ser/Thr phosphatase activities. As shown in Fig. 2c, WipB 1-364 clearly exhibits phosphatase activity. Biochemical quantification of the initial rates at 21 °C revealed a $K_m$ of 0.5 ± 0.3 mM and a $V_{max}$ of 0.042 ± 0.02 mM.s$^{-1}$ for the dephosphorylation reaction using 30 nM of WipB 1-364.

Since WipB and WipA are structurally similar and WipA exhibits tyrosine protein phosphatase activity, we next tested the activity of WipB against a phosphotyrosine-containing peptide (Fig. 2c). The model peptide END(pY)INASL (a peptide derived from the T cell phosphatase sequence and generally used to assay tyrosine phosphatase activities) was used as substrate in the presence of 0, 10, 30 and 90 nM of WipB 1-364 and the released free phosphate concentration was measured after incubation at 37 °C for 2 minutes (Fig. 2c). The same experiment was carried out using the RRA(pT)V A peptide for comparison. We observed that WipB 1-364 is able to release phosphate from the phosphothreonine, not the phosphotyrosine, peptide. Another phosphotyrosine-containing peptide routinely used to monitor tyrosine dephosphorylation was also used, with sequence DADE(pY)LIPQQG derived from the EGFR protein, and the lack of activity against phosphotyrosine-containing sequences was confirmed (data not shown).

In order to assess whether the active site identified from comparison with the structure of PP2B is responsible for the measured phosphatase activity, we expressed and purified three derivatives of WipB 1-364 each containing an individual point mutation within the putative active site: Asp118 to Ala (WipB 1-364;D118A; in the GDLHA motif) and Arg123 to Ala (WipB 1-364;R123A; in the GDELVDR motif) and His151 to Ala (WipB 1-364;H151A; in the NHG motif).
The three mutants exhibited a loss in phosphatase activity confirming that the catalytic domain predicted from our structure is indeed functional (Fig. 2c).

To understand the differences between WipB and WipA specificity, we compared the structure of their catalytic sites (Fig. S2e). Both are essentially similar. However, four residues differ in position: Arg185 (123 in WipA), Asp30 (31 in WipA), Asp396 (331 in WipA) and Asp310 (369 in WipA). In WipA, the first three are involved in catalysis while Arg369 of WipA was hypothesized to be involved in pTyr recognition. In WipB, Arg310 (equivalent of Arg369 in WipA) is conformationally restrained away from the active site and therefore cannot assume a role in substrate recognition. We hypothesize that this is the reason why WipB is inactive against pTyr-containing peptides.

Altogether, these data demonstrate that WipB harbours a functional N-terminal phosphatase domain that can hydrolyse Ser/Thr phosphorylated peptides in vitro and is catalytically independent from the C-terminal domain.

WipB locates to the lysosomal compartment and its C-terminal domain determines its cellular localisation. Having established that WipB is a Ser/Thr protein phosphatase, we next investigated the localisation of WipB when expressed in cultured mammalian cells.

Cultured HeLa cells were transiently transfected with plasmids encoding GFP fusions of WipB, WipB<sub>D118A</sub> and WipB<sub>1-364</sub> and WipB<sub>365-524</sub>. Scale bar, 10 μm. (b) HeLa cells expressing GFP-WipB (green) stained using an anti-LAMP-1 antibody (Magenta) after permeabilisation and fixation. Scale bar, 10 μm. (c) HeLa cells expressing GFP-WipB (green) were incubated with LysoTracker (red) for 15 min before fixation. Scale bar, 10 μm. (d) SDS-PAGE of fractions following co-immunoprecipitation of GFP, GFP-WipB or the indicated GFP-WipB derivatives from HeLa cell lysates using anti-GFP antibody and immunoblotting with anti-v-ATPase A, -v-ATPase B or LAMTOR1 antibodies. A cropped blot is here displayed and the corresponding full-length blot is included in the supplementary information. (e) SDS-PAGE of fractions following co-immunoprecipitation of v-ATPase A, v-ATPase B or LAMTOR1 from lysates of HeLa cells expressing GFP or GFP-WipB and immunoblotting with anti-GFP antibody. A cropped blot is here displayed and the corresponding full-length blot is included in the supplementary information.

Figure 3. WipB is targeted to lysosomes by its C-terminal domain where it interacts with components of the lysosomal nutrient sensing system (a) HeLa cells expressing GFP-fusion proteins of WipB, WipB<sub>D118A</sub>-WipB<sub>1-364</sub> and WipB<sub>365-524</sub>. Scale bar, 10 μm. (b) HeLa cells expressing GFP-WipB (green) stained using an anti-LAMP-1 antibody (Magenta) after permeabilisation and fixation. Scale bar, 10 μm. (c) HeLa cells expressing GFP-WipB (green) were incubated with LysoTracker (red) for 15 min before fixation. Scale bar, 10 μm. (d) SDS-PAGE of fractions following co-immunoprecipitation of GFP, GFP-WipB or the indicated GFP-WipB derivatives from HeLa cell lysates using anti-GFP antibody and immunoblotting with anti-v-ATPase A, -v-ATPase B or LAMTOR1 antibodies. A cropped blot is here displayed and the corresponding full-length blot is included in the supplementary information. (e) SDS-PAGE of fractions following co-immunoprecipitation of v-ATPase A, v-ATPase B or LAMTOR1 from lysates of HeLa cells expressing GFP or GFP-WipB and immunoblotting with anti-GFP antibody. A cropped blot is here displayed and the corresponding full-length blot is included in the supplementary information.
mammalian target of rapamycin (mTORC1) kinase complex at the lysosomal surface. LAMTOR1 is a phosphoprotein that links the Ragulator complex to the lysosomal surface, which is also involved in mTOR signalling.

Strikingly, GFP-WipB 365-524 exhibits a localization similar to GFP-WipB, demonstrating that the C-terminal domain is sufficient for lysosomal targeting.

To investigate whether the GFP-WipB puncta were targeted to any specific cellular compartment or whether GFP-WipB expression influenced the distribution or morphology of cellular organelles, GFP-WipB transfected cells were fixed and co-stained with markers characteristic of different subcellular compartments and organelles. GFP-WipB did not co-localise or disrupt the morphology of the Golgi apparatus (giantin), mitochondria (MitoTracker®) or the rough endoplasmic reticulum (calreticulin) (Fig. S4). However, GFP-WipB was strongly enriched around LAMP-1 positive compartments (Fig. 3b). Similarly, when transfected cells were loaded with LysoTracker® that specifically labels acidified compartments, LysoTracker-positive compartments also coincided with WipB puncta (Fig. 3c). In addition, when cells expressing GFP-WipB were loaded with TRITC-Dextran 10,000, which accumulates in endosomal compartments, partial co-localisation with the WipB puncta was also evident (Fig. S3d). Indeed, GFP-WipB could be frequently and reproducibly visualised as a defined ring of fluorescence surrounding LAMP-1 positive, LysoTracker-positive and TRITC-Dextran loaded compartments in transfected cells (Fig. 3c and S3d, zoom panels), indicative of protein recruitment. We next assessed the extent of colocalisation by calculating Manders coefficients to allow an unbiased evaluation of the degree of overlap between GFP-WipB and the various cellular markers. The resulting quantitative analysis strongly reinforced our qualitative observations, demonstrating statistically significant associations between WipB-GFP and LAMTOR1, WipB-GFP and TRITC-Dextran, and most strikingly between WipB-GFP and LAMP1, but not between WipB-GFP and giantin (Fig. S3e). Correspondingly, the reciprocal analysis confirmed identical relationships when co-localisation was considered with respect to the individual cellular markers and GFP-WipB (Fig. S3e). These data reveal that the Ser/Thr phosphatase WipB is recruited to acidified LAMP1 compartments, characteristic of lysosomes.

To establish whether the Ser/Thr phosphatase activity influenced WipB localisation, cells were equivalently transfected with the catalytically dead GFP- WipB D118A derivative. As with active GFP-WipB, GFP- WipB D118A was stable after expression (Fig. S3a), and adopted a localisation indistinguishable from GFP-WipB (Figs 3a, S3d and S3f), demonstrating that Ser/Thr phosphatase activity is not a determinant of WipB localisation in eukaryotic cells.

To identify the region of WipB responsible for lysosomal targeting, GFP fusions of the isolated catalytic domain (GFP-WipB1-364) and C-terminal domain (GFP-WipB365-524) were equivalently transfected. Analysis of protein expression by immunoblotting of transfected cell lysates showed that each derivative was stably expressed as a fusion of the expected molecular weight (Fig. S3a). GFP-WipB1-364 and GFP-WipB365-524 were then visualised following transfection of HeLa cells using confocal microscopy (Fig. 3a). In contrast to GFP-Wip-B, GFP-WipB1-364 distributed uniformly throughout the cytoplasm, mirroring the localisation of control GFP. Strikingly, GFP-WipB365-524 exhibits a localization similar to GFP-Wip-B, demonstrating that the C-terminal domain is sufficient for lysosomal targeting.

**WipB interacts with components of the eukaryotic LYNUS apparatus.** The targeting of the WipB Ser/Thr phosphatase to lysosomes by the C-terminal domain, suggested that WipB might target host phosphoproteins within the endo-lysosomal system. To identify potential host targets of WipB, we performed co-immunoprecipitation experiments with anti-GFP antibodies from transfected cell lysates, followed by mass spectrometry analysis, using HeLa cells expressing GFP-WipB, the catalytically dead derivative GFP-WipB D118A, or GFP alone as a control. The proteins identified under each condition were compared and purged of those interacting with GFP alone. The proteins remaining in the GFP-WipB and GFP-WipB D118A datasets were strikingly enriched for lysosomal proteins with 23% of the lysosomal membrane proteome represented amongst the data. Amongst the common targets, we identified lysosomal proteins present within the top 50 hits that are also predicted to contain Ser/Thr phosphorylation sites according to the UNIPROT database. This included flotillin-1, LAMTOR1 (p18), and two subunits of the vacuolar H+–ATPase (v-ATPase), subunits B and d1 (Table 2). Three of these four proteins are functionally linked, as LAMTOR1 and the two v-ATPase subunits interact as part of a larger macromolecular assembly termed the lysosomal nutrient sensing (LYNUS) apparatus that controls the mammalian target of rapamycin (mTORC1) kinase complex at the lysosomal surface. LAMTOR1 is a phosphoprotein that links the Regulator complex to the lysosomal surface, which is also involved in mTOR signalling and is activated via the v-ATPase. Therefore, we focussed on these three WipB interactors.

Firstly, we verified our mass spectrometry results by immunoblotting the anti-GFP immunoprecipitation samples using antibodies specific for subunits A and B of the v-ATPase and against LAMTOR-1 (Fig. 3d and Fig. S5a). These data show that GFP-WipB and GFP-WipB D118A, but not GFP alone, can indeed immunoprecipitate the three target proteins.
Secondly, equivalent immunoprecipitations with N-terminal GFP-WipB\textsubscript{1-364} and C-terminal GFP-WipB\textsubscript{365-524} were performed (Fig. 3d and Fig. 5a). While GFP-WipB\textsubscript{365-524} interacts with the two v-ATPase subunits and LAMTOR1, GFP-WipB\textsubscript{1-364} only binds the v-ATPase subunits. This reveals that while both domains of WipB can engage with the v-ATPase subunits, only the C-terminal domain interact with LAMTOR1.

Finally, we performed reverse co-immunoprecipitation using antibodies against the v-ATPase A and B subunits and LAMTOR1 (Fig. 3e and Fig. S5b). Cells were transfected with either GFP alone or GFP-WipB and lysates incubated in the presence of beads coupled to antibodies against the v-ATPase A or B subunits, or LAMTOR1. Immunoprecipitated proteins were then analysed by SDS-PAGE and Western blotting using anti-GFP antibody. As shown in Fig. 3e, all three proteins were able to pull-down GFP-WipB, but not GFP alone.

Altogether, these results show that the WipB Ser/Thr phosphatase targets key components of the host LYNS complex on the lysosome, when expressed in mammalian cells.

Discussion
In this study, we determined the structure and characterized the catalytic domain of the \textit{Legionella pneumophila} effector WipB. We showed that this domain belongs to the PPP family of eukaryotic phosphatases and has a Ser/Thr phosphatase activity \textit{in vitro}\textsuperscript{21}. Ser/Thr phosphatases regulate a wide number of processes in eukaryotic cells, including targeting signalling cascade components to membrane receptors\textsuperscript{14}. Their substrate(s) specificity often resides within their regulatory domains/subunits. Similarly, we showed that WipB has a specific subcellular localisation in HeLa cells controlled by its C-terminal domain, which targets it to lysosomes.

We demonstrated that WipB can interact with subunits of the v-ATPase and LAMTOR1 when expressed in cultured cells. The v-ATPase is a transmembrane complex that controls the acidification of cellular organelles, comprised of 17 subunits\textsuperscript{22}, which harbour several phosphorylation sites that regulate pump function\textsuperscript{23}. \textit{Legionella} may modulate the function of the v-ATPase since part of the LCV membrane is derived from the lysosomal compartment\textsuperscript{24,25}, yet the lumen remains at neutral pH\textsuperscript{26}. Indeed, the \textit{Legionella} effector SidK binds the v-ATPase regulatory subunit VatA, resulting in the inhibition of the complex and organelle acidification\textsuperscript{26}. As with \textit{wipB} mutants, a \textit{sidK} mutant did not exhibit defects in intracellular replication\textsuperscript{26}. Consequently, it is possible that redundant functions of SidK and WipB converge to repress the activity of the host v-ATPase through direct binding, promoting bacterial growth.

Recently, mTOR-driven metabolic reprogramming was found to be important for intracellular replication by \textit{Legionella}\textsuperscript{27}. An unknown T4S effector/s was proposed to activate mTOR to promote LCV expansion, a process that required a functional Dot/Icm secretion system and the Icm\textit{s} chaperone, in addition to host phosphatidylinositol-3-kinase. Lysosomes play an important role in coordinating nutrient sensing and signalling pathways involved in cell metabolism and growth. LAMTOR1 is a key phosphoprotein of this system\textsuperscript{20}, directing the nucleation of the multi-component LYNUS apparatus including mTOR and v-ATPase on the lysosomal surface\textsuperscript{27}, although its mode of regulation are not yet clearly understood. Although further investigation is required, it is tempting to speculate that WipB is a T4S effector involved in the metabolic reprogramming of \textit{Legionella}-infected cells.

Using transposon site hybridization (TraSH), several “functional groups” of effectors were proposed to co-concomitantly act on cellular pathways: while individual effector deletion has no effect, their combined deletion altered \textit{L. pneumophila} growth in host cells\textsuperscript{41}. While single deletion of \textit{wipB} and \textit{lidA} are phenotypically silent, a \textit{wipB}/\textit{lidA} double mutant exhibited defects in the stability of the LCV. During infection, LidA promotes the recruitment of ER-derived vesicles to the LCV through its binding to the AMPylated form of the Rab1 GTPase\textsuperscript{28}. In this manner, LidA contributes to replication-niche formation, essential for \textit{L. pneumophila} to gather the resources it needs for cell division. On the other hand, escaping the host defence mechanisms by repressing the lysosomal pathway, a process WipB could be involved in, appears crucial to bacterial survival. Thus, impairing both LidA and WipB function would alter two main complementary virulence strategies, eventually leading to intracellular growth defects.

A recent genetic study conducted on the genomes of 41 \textit{Legionella} species remarkably showed that effector repertoires share some conserved domains within and between species\textsuperscript{49}. Those domains, catalytic, binding or uncharacterized domains, are shuffled to generate effector pools, which consist of nearly 6000 effectors in the whole sequenced genus. Using the \textit{L. pneumophila} effectors, the authors define 608 orthologue groups of proteins sharing one or several conserved domains, representing 80% of the 6000 putative proteins. One orthologue group is based on the WipB sequence (group number LOG\textsubscript{001109}), which according to their findings, could have orthologues in 32 out of the 41 sequenced \textit{Legionella} species. In addition, a search of homologues to the WipB catalytic domain using BLAST\textsuperscript{50} also identifies hypothetical proteins effectors in \textit{Coxiella} and \textit{Fluoribacter} species that could then share the same enzymatic activity. Thus our findings on \textit{L. pneumophila} WipB might have implications for understanding a whole family of effectors from the entire \textit{Legionella} genus.

\textit{L. pneumophila} secretes a wide range of protein effectors during the infection that leads to Legionnaire’s disease. Assigning the functions of these effectors is often complex given the high redundancy they exhibit. Here we demonstrate how combining structural biology and cellular biology is a powerful alternative route to identifying the role and targets of \textit{Legionella} effectors.

Methods
Cloning. The WipB DNA (AAU28775) encoding the wild type protein (Q5ZS02\_LEGPH, lpg2718) was cloned in a modified pETM14 vector (EMBL) or in empty pEGFP-C2 and -N2 vectors using a PCR-based in-fusion HD cloning system (Clontech Laboratories). For the pETM14-derived constructs, the expression cassette contained an N-terminal deca-histidine tag followed by a 3 C protease cleavage site. Site directed mutagenesis was performed using standard molecular biology protocols.
Expression and purification of WipB\textsubscript{25-344} and WipB\textsubscript{1-364}. All recombinant proteins were over-expressed in C43(DE3) bacterial strains. The cells were harvested by centrifugation (6000 g, 15 min) and resuspended in a lysis buffer containing 25 mM Tris-HCl pH 7.5, 0.3 M NaCl, 5 mM β-mercaptoethanol (βME), 10 mM imidazole, 5% glycerol, a tablet of protease inhibitors (Complete, EDTA-free by Roche) and 0.25 mg/ml lysozyme. Cells were lysed in an EmulsiFlex-C3 homogeniser (Avestin) and the crude extract was centrifuged at 50,000 g for 45 min. The supernatant was loaded onto a 5 ml HisTrap column (GE Healthcare) equilibrated with the lysis buffer, connected on an AKTA purifier (GE Healthcare). Washing steps were performed with extended volumes of lysis buffer though the column as well high salt buffer (25 mM Tris-HCl pH 7.5, 1 M NaCl, 5 mM βME, 10 mM imidazole, 5% glycerol) at a molar ratio protein/protease of 50:1. The cleaved protein was collected in the flowthrough of two 1 ml HisTrap and GST-Trap columns (GE Healthcare) connected in sequence to remove uncleaved proteins and the protease. It was then loaded on a resource Q column (GE Healthcare) and eluted with a gradient of NaCl at a concentration of about 0.1 M NaCl. The eluted protein was further concentrated and loaded to a superdex200 16/60 column (GE healthcare) equilibrated in 25 mM Tris-HCl pH 7.5, 0.15 M NaCl, and 5% glycerol.

Phosphatase activity assay. Assays were conducted on purified protein after His-tag removal using the Tyrosine and Ser/Thr Phosphatase Assay Systems kits (Promega). Briefly, the protein was diluted in its purification buffer containing 1 mM of MnCl\textsubscript{2} and incubated at either 21 °C or 37 °C with the provided peptide before stopping the reaction with a buffer containing molybdate and malachite green to measure free phosphate at 600 nm in a 96-well microplate. One phosphotyrosine-containing peptide (END(pY)INASL and DADE(pY)LIPQQG) were provided in the kits and were used as per manufacturer’s instructions.

Crystalization, data collection and processing. Initial crystallization screens were performed using the sitting-drop vapor-diffusion technique, by mixing equal volumes (0.2 μl) of protein solution (13 mg/ml) at 16 °C. Crystals in the shape of thin needles appeared after 3-4 days reaching a maximum length of 0.1-0.2 mm against a reservoir solution containing 0.23 M ammonium citrate dibasic and 22% PEG3000. Before data collection, harvested crystals were immersed in a solution containing the precipitant mixture and 10% MDP and cryo-cooled in liquid nitrogen. All data sets were collected at 100 K. Crystals of the WipB\textsubscript{25-344} were collected at the PetraIII P13 beam-line (EMBL-Hamburg/DESY P13, Germany). The data set was indexed, processed and scaled using the XDS package\textsuperscript{31}, (Table 1).

Structure determination and refinement. The WipB\textsubscript{25-344} crystals belonged to the P 2\textsubscript{1} space group with a solvent content of 43.12% corresponding to two molecules per asymmetric unit (AU). The structure was determined by molecular replacement using Molrep\textsuperscript{32} and the WipA structure (PDB Code 5N72) containing only the α-helices and β-strands of the phosphatase domain as search model. The coordinates were further improved by maximum-likelihood and TLS refinement using the PHENIX suite\textsuperscript{33} and manual improvements of the model using COOT\textsuperscript{34}. The final model converged to a final R\text{work}/R\text{free} of 0.187/0.217 at a resolution of 1.70 Å. The WipB\textsubscript{25-344} model covers the WipB amino-acid sequence 24-331 (chain A) and 25–344 (chain B) and contains in addition 484 water molecules.

Antibodies and cellular biology reagents. CHIP grade rabbit anti-GFP (Invitrogen) was used for co-immunoprecipitation and mouse anti-GFP (clontech) for Western-blotting. Rabbit anti-vATPase A (Abcam), anti-vATPase B (Abcam) or anti-LAMTOR1 (Sigma) were used for co-immunoprecipitation and Western blotting. Anti-Sec. 61b (Millipore) was used as a loading control in Western-blotting. HRP-coupled anti-mouse and anti-rabbit (Abcam), rabbit anti-vATPase A (Abcam), anti-LAMTOR1 (Sigma) or anti-LAMTOR1 antibodies in a buffer PBS Tween 0.02% (v/v) 10% BSA (w/v) for 10 min. Cell lysates were incubated with the washed beads for 1 h in the presence of 5% BSA to reduce non-specific interactions. After washing, proteins were eluted with 75 mM Tris HCl pH 7.4, 0.5 M EDTA, 0.05% SDS and analyzed by western blotting.
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Author Contributions
M.S.P. performed the biochemistry and cellular experiments and some of the crystallography experiments; M.D. and M.S.P. analysed the cellular data; N.P. designed the biochemical experiments, purified, crystallized and solved the structure; G.W. and R.D.H. supervised the research; and all authors wrote the paper.

Additional Information
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