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Supporting information for article:

Structural basis for the acetylation mechanism of the *Legionella* effector VipF

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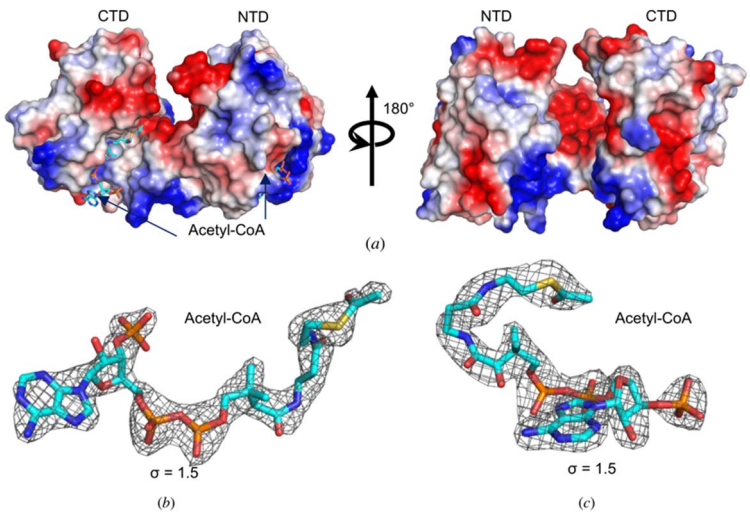

Figure S1 Conformations of acetyl-CoA in its complex structure with VipF. (*a*) and (*b*) Simulated omit map of acetyl-CoA contoured at 1.5 σ. The acetyl-CoAs are from VipF's CTD (*a*) and NTD (*b*), respectively. The electron density map is shown as a grey mesh, and the acetyl-CoA is depicted as a stick model. (*c*) Surface representation of VipF in the complex with acetyl-CoA, which is colored by electrostatic surface potentials [contoured from −5kBT (red) to +5kBT (blue)]. Acetyl-CoA molecules are shown as sticks (cyan).

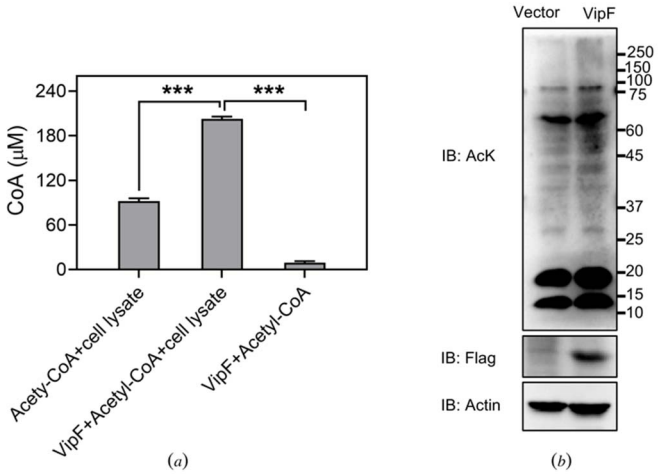

Figure S2 Potential substrates in mammalian cells for VipF. (*a*) Total cell lysate of HEK293T cells as a substrate for VipF. The total lysate was prepared using the HEK293T cells grown at 80% confluency in 12-well plate and lysed using freeze-thaw cycles. Purified recombinant VipF protein was used at a working concentration of 400 μg/mL for 500 μg lysate protein. Data are a mean ± SEM, n=3. ***, $p < 0.001$ (Student's *t* test). (*b*) Total protein acetylation with ectopic VipF expression. HEK293T cells transfected with a plasmid pCMV-3×Flag-VipF for 24 h were lysed by 1.25× SDS-loading buffer and separated by a 12% SDS-PAGE gel. VipF was determined by anti-Flag antibody (Proteintech, China). Total protein acetylation was analyzed by Western blotting using anti-acetyl-lysine antibody (PTM Biolabs, China) with an empty vector as a control, and β-actin was used as protein loading control. Protein markers (kDa) are labeled on the right.

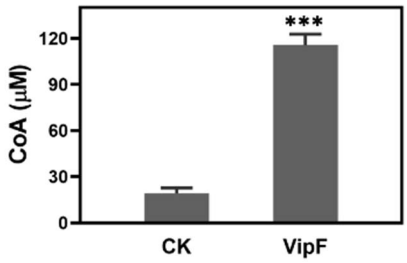

Figure S3 VipF acetyl-transferase activity using chloramphenicol, a putative substrate. The activity was measured by CoA production. CK was a reaction without VipF. Data are shown as a mean of three measurements. ***, $p < 0.001$ (Student's *t* test).

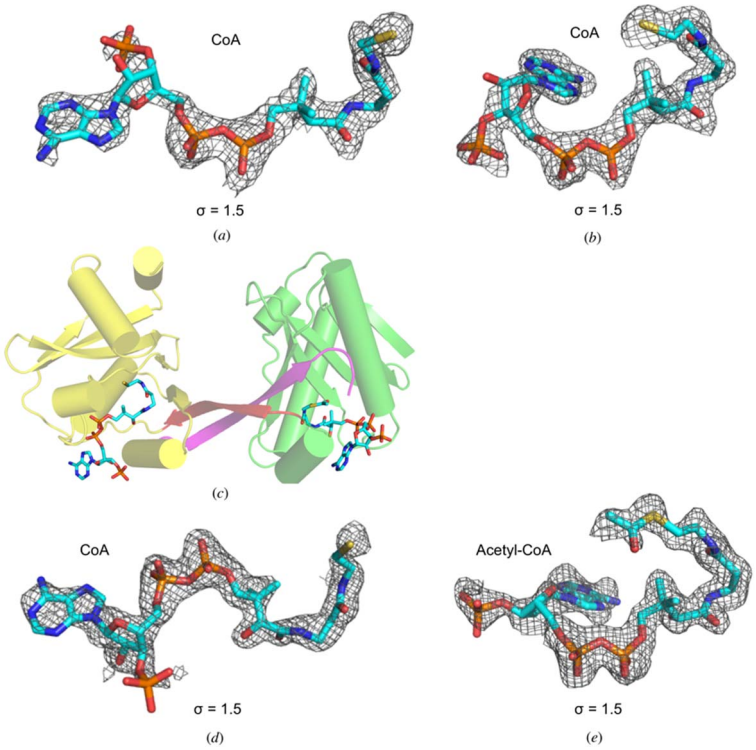

Figure S4 Electron densities of acetyl-CoA/CoA complexed in the VipF structures crystallized in presence of chloramphenicol. (*a*) and (*b*) Simulated omit maps of CoA in a complex structure of VipF/CoA/ chloramphenicol. CoAs bound in CTD (*a*) and NTD (*b*) are depicted as stick models. **C.** Overall complex structure of VipF/acetyl-CoA/CoA. (*d*) and (*e*) Simulated omit maps of CoA bound in CTD (*d*) and acetyl-CoA bound in NTD (*e*). All electron density maps contoured at 1.5 σ are in the grey mesh. Acetyl-CoA/CoA are shown as stick models.

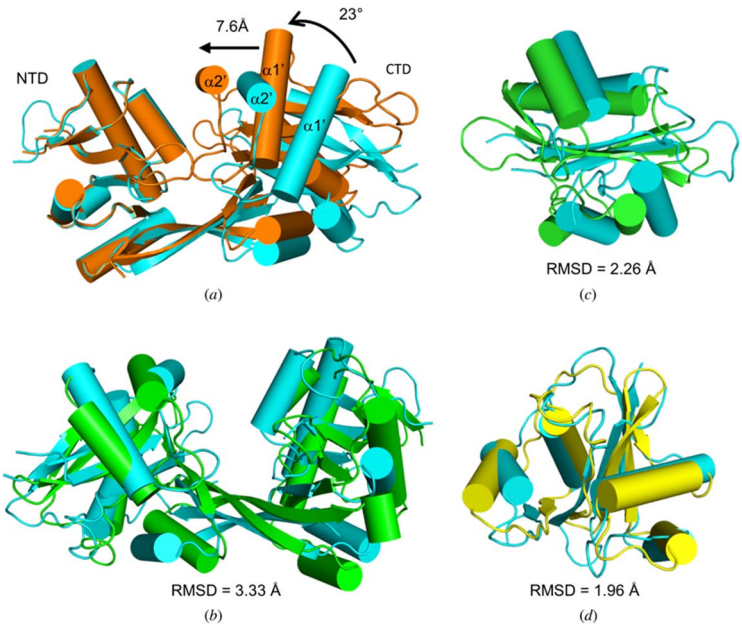

Figure S5 Comparison of the VipF structure with MshD. *a*. Conformational change of *Mycobacterium tuberculosis* mycothiol synthase MshD in substrate free and bound states (PDB 1OZP, free state in cyan and 2C27, bound state in orange). The two structures were aligned using their NTDs. *b*-*d*. Comparison of VipF to the open MshD conformation in a substrate-free state. Overall alignment shown in *b*, NTD in *c*, and CTD in *d*. VipF is colored in green, and MshD in cyan. The MshD CTD in *d* is colored in yellow.

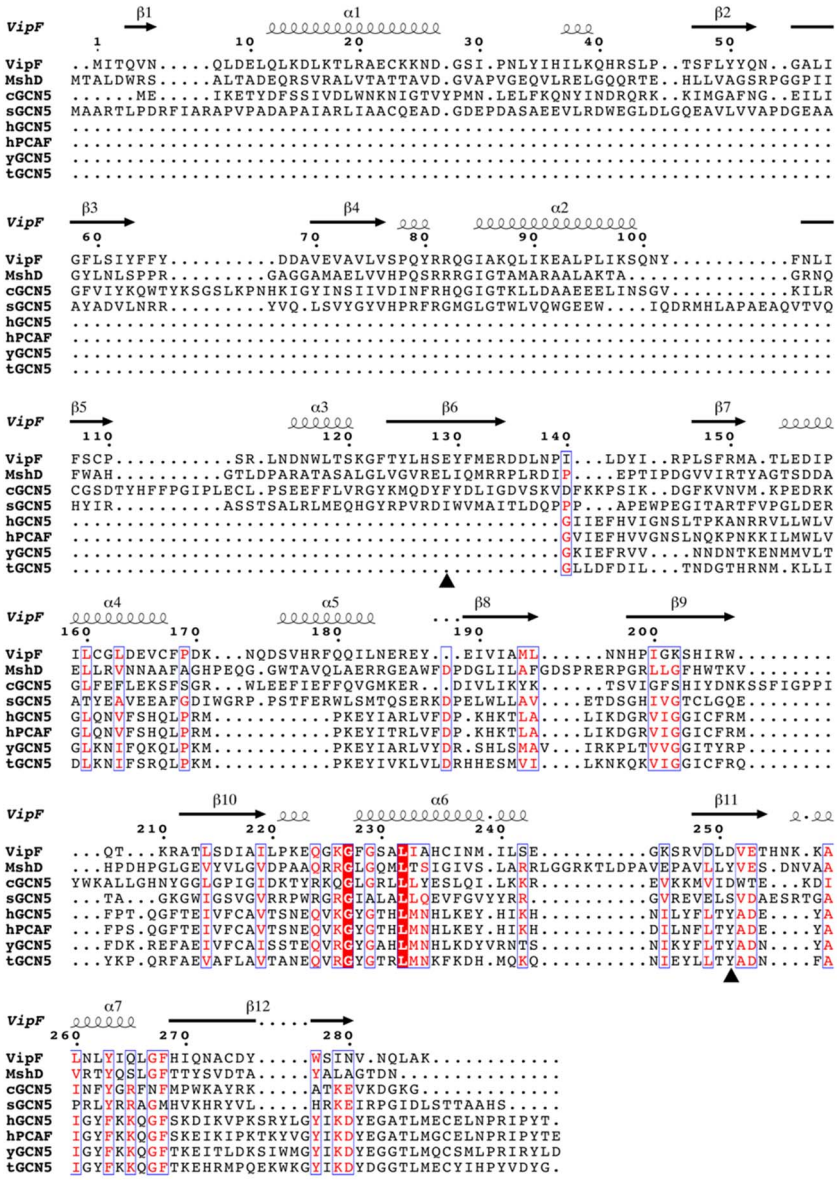

Figure S6 Structure-based sequence alignment of VipF to several well-defined acetyltransferases. The sequence alignment was performed by PROMALS3D and produced in ESPript server. The conserved residues are shown in red, and completely conserved residues in white over a red background. The secondary structural information and residue numbering of VipF are labeled on top. MshD: *M. tuberculosis* mycothiol synthase (PDB ID:1OZP); cGCN5: *Clostridium acetobutylicum* Glucosamine/glucosamine N-acetyltransferase (PDB ID:5KF9); sGCN5: *Sphaerobacter thermophilus* GCN5-related N-acetyltransferase (PDB ID:3TT2); yGCN5: Yeast general control non-derepressible 5-related N-acetyltransferase (PDB ID:1YGH); hGCN5: Human general control non-derepressible 5-related N-

acetyltransferase (PDB ID:1Z4R); tGCN5: *Tetrahymena thermophila* GCN5 (PDB ID:1QST); hPCAF: Human p300/CBP-associated factor (PDB ID:1CM0). The positions of Glu129 and Asp251 are indicated by black triangles.

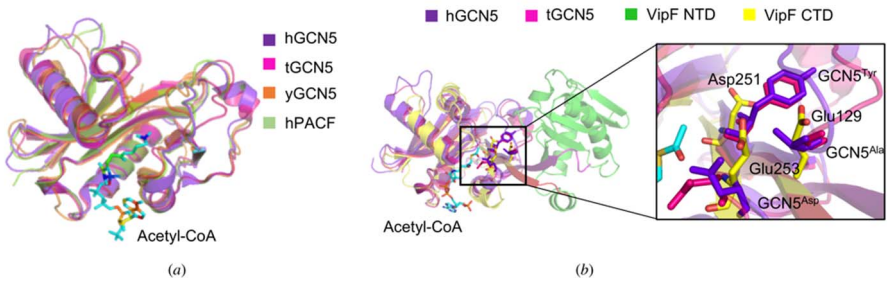

Figure S7 Structural alignment of VipF with the GCN5 family members. **(***a***)** Structural alignment of GCN5 fmaily members, including hGCN5 (puple), tGCN5 (hot pink, RMSD=0.714Å), yGCN5 (orange, RMSD=0.785Å) and hPCAF (chartreuse, RMSD=0.414 Å). (*b*) The highlighted active site of the GCN5 family. The residues Glu129 and Asp251 in the active site of VipF and corresponding residues in the GCN5 family are shown in stick models. The RMSD of hGCN5 and tGCN5 are 0.333 Å and 2.075 Å, respectively.The NTD is shown in green; The CTD is in yellow with its acetyl-CoA in cyan.

Table S1 Oligonucleotide primers used in this study

Primers	Sequences (5'→3')
VipF-F	ATGTTACTCAAGTTAACC
VipF-R	TTATTTTGCAAGTTGATTCAC
N32A-F	CCCCGCACTATATATCCATATATTGAAACAACAC
N32A-R	GGATATATAGTGCGGGGATGCTGCCATC
L33A-F	CCAATGCATATATCCATATATTGAAACAACACC
L33A-R	GGATATATGCATTGGGGATGCTGCC
Y34A-F	CAATCTAGCAATCCATATATTGAAACAACACC
Y34A-R	GGATTGCTAGATTGGGGATGCTGCCATC
E129A-F	CATAGCGCATATTTCATGGAACGTGATG
E129A-R	GAAATATGCGCTATGCAGGTAGG
D251A-F	CCTTGCAGTTGAAACCCATAATAAAAAAGC
D251A-R	GTTTCAACTGCAAGGTCCACCCGGG