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Supporting Online Material for

De-AMPylation of the Small GTPase Rab1 by the Pathogen *Legionella pneumophila*

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MATERIALS AND METHODS

Strains, media, and plasmids

L. pneumophila strains were grown and maintained as described (*21, 22*). *L. pneumophila* strains Lp02 (*thyA hsdR rpsL*) and Lp03 (Lp02 *dotA3* (T4SS$^-$)) are thymidine-auxotroph derivatives of Philadelphia-1 (*23, 24*). *L. pneumophila* mutants with in frame deletions in *sidD* or *lepB* were generated by allelic exchange as described (*14*). Plasmids were introduced into *L. pneumophila* by natural transformation (*25*). Infectious center assay and growth curve analysis were performed as previously described (*19, 26*).

Plasmids for production of recombinant tagged-proteins in *Escherichia coli* and of fluorescently tagged proteins in tissue culture cells are listed in Table S1. The oligonocleotides used to PCR-amplify the desired DNA fragments and the restriction sites used to clone the open reading frames are listed in Table S2. Plasmids created in this study were cloned as described in Table S1. Antibodies were purchased from Santa Cruz Biotechnology (Rab1B, Rab5), Covance (Giantin), Abd Serotec (TGN46), and Abcam (Cox1). Antibodies directed against LepB (GenScript) and SidD (Covance) were generated in rabbit by immunizing with purified recombinant full length protein according to standard protocols. Antibodies directed against *L. pneumophila* were generated in rabbit as well as rat using formalin-killed bacteria. Anti-ICDH antiserum was kindly provided by Abraham (Linc) Sonenshein (Tufts University, Boston). Anti-SNX2 antiserum was a kind gift from Juan Bonifacino (NICHD/NIH, Maryland). Anti-SidM antibody was described previously (*6*).

Production and purification of recombinant proteins

Plasmid constructs were expressed in *E. coli* BL21 at 20°C over night after induction with 0.2 mM isopropyl-β-dithiogalactopyranoside (IPTG). Tagged proteins were purified from the soluble fraction of the bacterial lysate using either GE Healthcare's Glutathione Sepharose 4B slurry for GST-tagged proteins or TALON Metal Affinity Resin (Clontech) complexed with cobalt for 6×His-tagged proteins. The glutathione sepharose was pre-equilibrated in PBS supplemented with 1 mM $MgCl_2$ and 1 mM β-mercaptoethanol (β-ME) (PBS-MM). Binding of the GST-tagged proteins was performed at 4°C over night. Protein-bound beads were washed with the equilibration buffer and either eluted with 50 mM Tris-HCl (pH 8) containing 10 mM reduced glutathione (GE Healthcare) or were incubated over night with PreScission protease to cleave off the GST tag. Tagged or untagged protein eluted from the beads was dialyzed over night in equilibration buffer and stored in multiple aliquots at -80°C. For purification of His-tagged proteins, the cobalt resin was pre-equilibrated in PBS supplemented with 1 mM $MgCl_2$, 1 mM β-ME, and 10 mM imidazole. His-tagged proteins were allowed to bind to the resin over night at 4°C. The resin was then washed and bound proteins were eluted in PBS supplemented with 125 mM imidazole. His-tagged proteins were dialyzed over night in PBS-MM and stored in multiple aliquots at -80°C. For experiments performed in phosphate-free buffer, proteins were purified in 40 mM Tris-HCl (pH 7.5) supplemented with 1 mM $MgCl_2$, 1 mM β-ME (Tris-MM) buffer instead of PBS.

In vitro AMPylation of Rab1a and purification of Rab1a-AMP

Rab1a was AMPylated at room temperature for 4 h in the presence of 2.5 molar excess of ATP and GTP and a 1:5 molar ratio of SidM to Rab1a in PBS containing 1 mM β-ME. Rab1a-

AMP was purified by gel filtration on a HiLoad 16/60 Superdex 75 pg (GE Healthcare) at 4ºC. Fractions containing Rab1a-AMP in either PBS or phosphate-free 40 mM Tris-HCl (pH 7.5) were pooled, concentrated, and stored at -80°C.

De-AMPylation assays (dose-curve)

Rab1a-[^{32}P]AMP (10 μM) was obtained in a reaction catalyzed by 7×10^7 SidM-coated beads (Dynabeads M-270 Epoxy, Invitrogen) in the presence of 12.5 nM [α^{32}P]ATP, 10 μM cold ATP, and 50 μM cold GTP in PBS or 40 mM Tris-HCl (pH 7.5). After removal of SidM-coated beads, de-AMPylation was initiated by addition of 10 μM Rab1a-[^{32}P]AMP to reactions containing either 100 μg lysate if not otherwise indicated or increasing concentrations of purified GST-SidD (1 nM, 2 nM, 10 nM, 20 nM, and 100 nM). Loss of [^{32}P]AMP from Rab1 was monitored by nitrocelluloase filter-binding assays as described for GTP hydrolysis.

Specific enzymatic activity of SidD

The specific enzymatic activity of SidD was determined using 10 μM Rab1a-[^{32}P]AMP in PBS in the presence of 12.5 nM [α^{32}P]ATP, 10 μM cold ATP, 50 μM cold GTP, and 20 nM GST-SidD. Rab1a-[^{32}P]AMP levels were monitored as described for GTP hydrolysis. Initial velocity (5 minutes) of [^{32}P]AMP loss from Rab1 was used to calculate specific enzymatic activity. The specific enzymatic activity estimate may be somewhat higher than we report here. This is due to the fact that our calculations did not account for the presence of GTP in the AMPylation reaction, which can be used by SidM to GMPylate Rab1, though ATP was shown to be the preferred substrate for SidM (*3*).

AMPylation/de-AMPylation cycles

Three reactions (A, B, and C) containing Rab1a (10 μM) loaded with cold GTP as described below were supplemented with 50 nM [α^{32}P]ATP, 30 μM cold ATP, and 10 μM GTP in PBS in 300 μl total volume. Samples A, B, and C were subjected to three, two, or one round(s) of AMPylation/de-AMPylation, respectively. Each round consisted of a 60-minute AMPylation reaction catalyzed by SidM-coated beads, followed by a 30-minute de-AMPylation reaction catalyzed by SidD-coated beads. In the first round, sample A was incubated with SidM-coated beads, while samples B and C were incubated with GST-coated control beads. After 60 minutes, the reactions were transferred to tubes containing SidD-coated beads for sample A, and GST-coated beads for samples B and C. In the second round, samples A and B were incubated with enzyme-coated beads, while sample C was incubated with GST-coated beads. In the third round, all three were incubated with enzyme-coated beads. 12 μl aliquots were removed at the indicated time-points to measure radioactivity using the nitrocellulose-binding assay.

Generation of *L. pneumophila* lysate

L. pneumophila wild-type (Lp02) or *ΔsidD* mutant were grown in liquid culture to late exponential phase. Cells were harvested by centrifugation, resuspended in PBS +1 mM β-ME 5 μl DNase (NEB), EDTA-free protease inhibitor cocktail (Roche) and lysed either by French press at 20,000 psi or sonication. Whole-cell lysate was cleared by centrifugation at 3000×g for 20 minutes and the supernatant was subsequently used for de-AMPylation assays.

Phosphodiesterase assay

cAMP phosphodiesterase assay using PDE8A (SignalChem) (25 ng, 50 ng, 100 ng - positive

control) and His-SidD (175 ng, 350 ng, and 700 ng) in the presence of 100 μM cAMP was performed using PDE-Glo™ Phosphodiesterase Assay Kit (Promega).

AMP and ADP detection assays

Rab1a-AMP (5 μM) was de-AMPylated in PBS or 40 mM Tris-HCl (pH 7.5) supplemented with 10 mM $MgCl_2$, and 0.1 mg/ml BSA by 0.3 μM GST-SidD purified using size-exclusion chromatography. De-AMPylation was allowed to proceed for one hour at room temperature. A competitive antibody-based fluorescence polarization assay (*Transcreener*® AMP assay, BellBrook Labs) was used to detect AMP released in the reaction according to manufacturer instructions. Briefly, the detection mix was comprised of 14.6 μg/ml anti-AMP polyclonal antibody, 4 nM AMP/GMP Alexa633 Tracer in buffer containing 20 mM HEPES (pH 7.5), 40 mM EDTA, and 0.02% Brij-35. AMP standards served as positive controls for detection.

For ADP detection, samples from the same reactions that were used to measure AMP, were analyzed using an indirect assay based on luminescence (ADP-Glo™ Kinase Assay, Promega). ADP standards were included in the experiment for comparison.

Detection and quantification of luminescence or fluorescence polarization were performed using the Synergy™ 4 Microplate Reader (BioTek Instruments).

GDP/[γ^{32}P]GTP exchange assay

[γ^{32}P]GTP incorporation studies were performed as described (*6*). Briefly, Rab1a and Rab1a-AMP were subjected to extraction of $MgCl_2$ by incubation with 5 mM ethylenediaminetetraacetic acid (EDTA) for 10 minutes. Proteins were then incubated in the presence of [γ^{32}P]GTP for 1h at room temperature followed by addition of 20 mM $MgCl_2$.

GTP hydrolysis assays

1 μM Rab1a or Rab1a-AMP loaded with [γ^{32}P]GTP was incubated at room temperature in PBS-MM containing 40 nM of either $LepB_{1232}$ (amino acid 1-1232) or $TBC1D20_{364}$ (amino acid 1-364). At the indicated time points, samples were transferred to PBS-MM containing 20 mM $MgCl_2$ and immediately applied to a pre-wet nitrocellulose filter (0.45 μm-Millipore, HAWPO2500, Millipore) on a vacuum filtration manifold (Millipore, model 1225). Filters were washed with 2 ml of PBS-MM. Nitrocellulose filters were transferred to vials containing scintillation liquid and radioactivity was measured by Beckman LS 6500 liquid scintillation counter.

For GAP assays of de-AMPylated protein, Rab1a (2 μM) was simultaneously activated and AMPylated by SidM-coated beads using [γ^{32}P]GTP (25 nM) and ATP (40 μM), respectively, in PBS-MM. Samples were removed at various time points to monitor saturation of nucleotide exchange using nitrocellulose filter-binding as described above. After 90 minutes, 1/5 of the mixture was added to a reaction containing either GST-SidD (0.35 μM) and GAP (70 nM), or GAP alone. The sample volume removed at each time point to monitor GTP hydrolysis was adjusted to account for the 5× dilution of the input.

Immunofluorescence microscopy

COS-1 cells were transiently transfected with pEGFP or pmCherry constructs using Lipofectamine 2000 Transfection Reagent (Invitrogen). Cells were fixed in PBS with 3.7% formaldehyde for 20 min at 37°C, permeabilized, blocked in PBS +10% goat serum, and stained for the indicated proteins as described (*6*). Nuclear DNA was labeled using Hoechst stain

(Invitrogen). Coverslips were mounted using Anti-fade reagent (Invitrogen).

Primary bone marrow-derived macrophages from A/J mice (Jackson Laboratories) were isolated as described (*27*). Macrophage monolayers were challenged with the indicated *L. pneumophila* strains at an MOI=1. At the indicated time points cells were fixed using 3.7% formaldehyde in PBS, blocked with PBS + 10% goat serum, and stained for extracellular bacteria using rat anti-*Legionella* antibody and goat anti-rat Cascade Blue. Membranes were permeabilized for ten seconds using -20ºC methanol, blocked, and stained for bacteria using rat anti-*Legionella* primary antibody and secondary goat anti-rat Texas Red antibody. Rab1 was detected by affinity-purified rabbit anti-Rab1B antibody and goat anti-rabbit FITC.

Digitonin fractionation of U937 cells infected with *L. pneumophila*

The digitonin fractionation assay was performed as previously described (*19*). Briefly, differentiated U937 cells ($1x10^7$) in 10 cm dishes were incubated with postexponential *L. pneumophila* wild-type or Lp03 (T4SS-defective mutant) at MOI=5. Cells were resuspended in 500 µl PBS containing 1% digitonin and incubated for 20 min at room temperature with vortexing. The cell lysate was centrifuged for 15 min at 10,000 rpm and the digitonin soluble fraction was suspended in sample buffer and analyzed by SDS-PAGE followed by Western blot analysis.

Expression profile in liquid culture

A fresh patch of *L. pneumophila* was resuspended in AYET media and used to create a two-fold serial dilution (seven culture tubes total). Bacteria were grown over night (1st cycle), samples were taken, the OD_{600} was determined, and the bacteria were resuspended in SDS-PAGE buffer. Bacteria from stationary growth phase were diluted into fresh AYET medium to an OD_{600}=0.05, and bacterial growth was monitored for an additional 24 hours (2nd cycle). Samples were taken in two-hour intervals to determine OD_{600}, and the bacteria were resuspended in SDS-PAGE buffer. Equal amounts of proteins were separated by SDS-PAGE and detected by Western blot using antibodies against SidD, LepB, or SidM. Isocitrate dehydrogenease (ICDH) was used as loading control.

Mass spectrometry

Gel-separated proteins were digested over night *in situ* using trypsin (0.02 mg/mL in 0.1M $NH_4H_2CO_3$). Peptides were recovered from digest mixture, salts were removed using C_{18} ZipTips and eluted into 10 uL 1:1 acetonitrile (ACN) and 0.1% trifluoacetic acid. A 0.5 µL aliquot of peptides from each sample were spotted into sample wells along with an equal volume of matrix (CHCA, 0.5 mg/mL) in a 1:1 solution of ACN:0.1% TFA that was 0.1M in $NH_4H_2PO_4$ and contained two peptides as internal standards. Mass spectra, 400 laser shots, were obtained using an ABI 4800 MALDI TOF/TOF and up to 5 selected peptides fragmented in gas-off mode using 1000 laser shots per peptide. Spectral data were recovered to a local computer from the mass spectrometer's Oracle database using the GUIExtractor Java routine for subsequent analysis. Protein identifications were undertaken using Mascot and confirmed using ProteinProcessor, an in-house Perl script that leads to increased protein sequence coverage.

SUPPORTING FIGURES

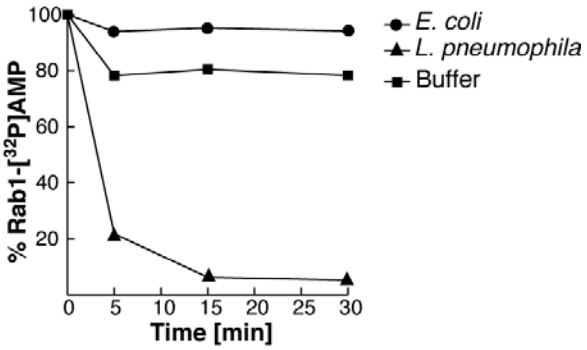

Fig. S1. *L. pneumophila* but not *E. coli* lysate contains a Rab1 de-AMPylase. De-AMPylation of Rab1-[^{32}P]AMP (1 μM) by *E coli* or *L. pneumophila* lysate (160 μg each). The amount of Rab1-[^{32}P]AMP was monitored by filter-binding assay. The graph is a representative of three independent experiments.

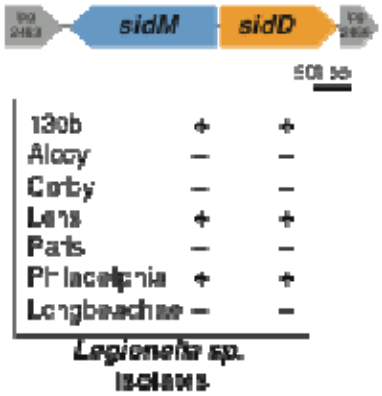

Fig. S2. Synteny of *sidM* and *sidD*. Schematic representation of the genomic organization of *sidD* and *sidM* in *L. pneumophila* strain Philadelphia-1. The table summarizes the presence (+) or absence (-) of *sidD* and *sidM* in the sequenced *Legionella* genomes.

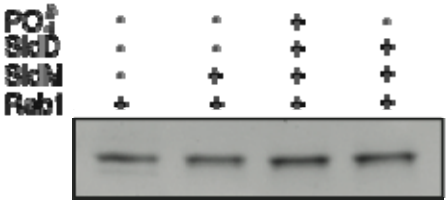

Fig. S3. Rab1 is not degraded by SidD. Rab1 AMPylated by SidM was incubated with SidD in buffer with (+) or without (-) orthophosphate (PO_4^{2-}). Rab1 was visualized by SDS-PAGE and Coomassie staining. Protein bands shown in this gel were excised and analyzed by mass spectrometry (Fig. 2A main text).

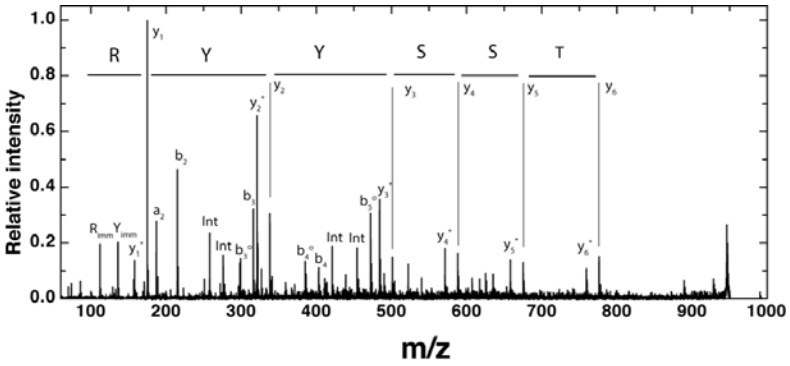

Fig. S4. De-AMPylation by SidD restores the primary sequence of Rab1. MALDI TOF/TOF fragmentation spectrum of the TITSSYYR peptide, m/z 990, of Rab1a following de-AMPylation (see Fig. 2A). Spectrum shows extensive y-series fragment ions as well as a number of b-series and other ions confirming the sequence of the peptide.

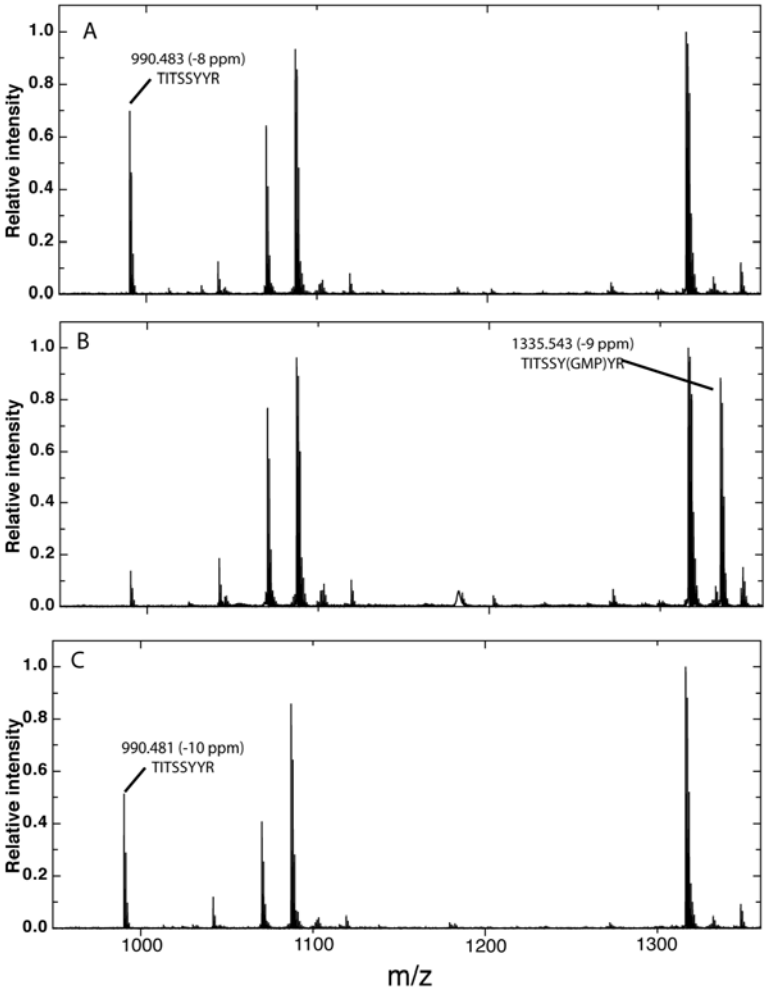

Fig. S5. SidD catalyzes de-GMPylation of Rab1. MALDI-TOF reflector spectra of a tryptic digest of Rab1a following de-GMPylation. Mass errors of labeled peptides are shown in ppm relative to predicted values. A) Unmodified Rab1A. B) Rab1a with GMPylation showing loss of intensity of TITSSYYR, m/z 990, peptide and existence of GMPylated peptide at m/z 1335. C) Rab1a following de-GMPylation. Peptide at m/z 1335 disappears and m/z 990 peptide reappears.

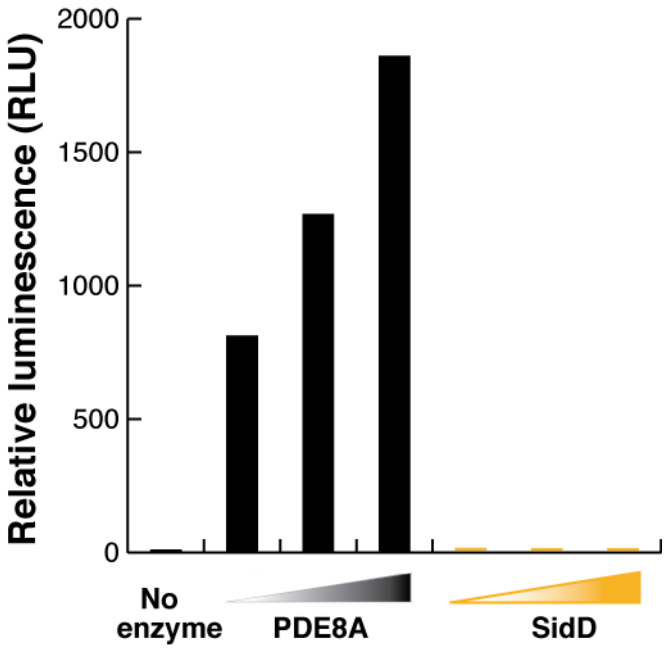

Fig. S6. SidD has no phosphodiesterase activity towards cAMP. Addition of AMP (or GMP) to tyrosine side chains creates a phosphodiester bond similar to that found in cyclic AMP (cAMP), a substrate of phosphodiesterases (PDEs). A promiscuous PDE present in snake venom can remove AMP from Rho GTPases AMPylated by IbpA from the pathogen *Histophilus somni* (*2*). cAMP was incubated with PDE8A (SignalChem) (25 ng, 50 ng, 100 ng - positive control) and His-SidD (175 ng, 350 ng, and 700 ng). The amount of cAMP hydrolyzed was determined by indirect luminescence.

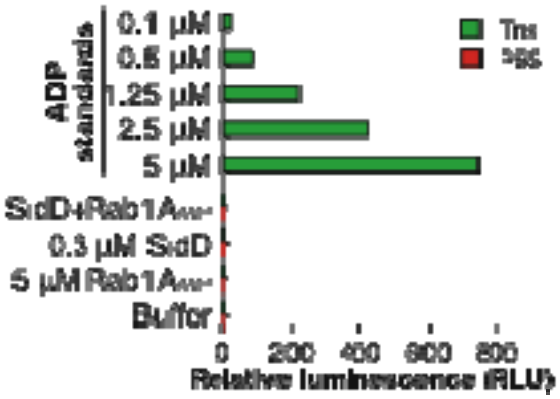

Fig. S7. SidD-catalyzed de-AMPylation of Rab1A does not generate ADP. The amount of AMP released from 5 µM Rab1-AMP incubated with 0.3 µM GST-SidD in the presence (PBS) or absence (Tris) of phosphate was detected by a competitive fluorescence polarization immunoassay. Detection of ADP standards in Tris buffer only is shown for comparison.

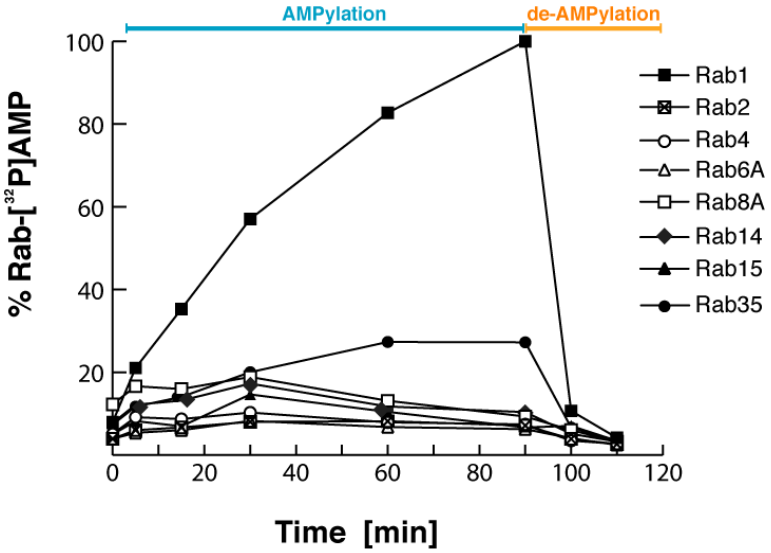

Fig. S8. Specificity of SidD de-AMPylation towards Rab GTPases. Purified glutathione *S*-transferase-tagged Rab GTPases (0.5 μM) loaded with GTP served as a substrate for AMPylation catalyzed by SidM (100 nM) in the presence of [α^{32}P]ATP (50 nM). After incubation with SidM and [α^{32}P]ATP, only Rab35 showed an intermediate level of AMPylation (<25%) compared to Rab1, suggesting that discrimination between different Rab GTPases by SidM may be more stringent than previously reported (*3*). De-AMPylation was initiated after 90 minutes by addition of 1 μM His-SidD and 100 μM cold ATP. Samples taken at the indicated time points were analyzed by filter-binding assay to monitor the levels of Rab1-[^{32}P]AMP.

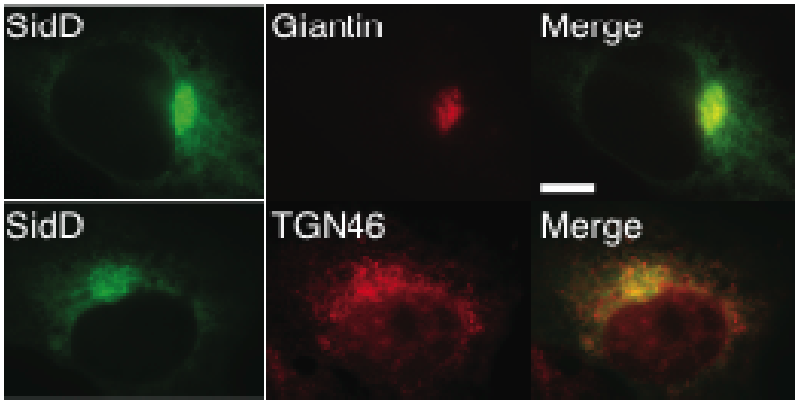

Fig. S9. SidD colocalization with Golgi compartments. Transiently transfected COS1 cells producing GFP-SidD (left panels) were fixed and stained for the indicated organelle marker (middle panels). The merged images (right panels) show SidD in green and the organelle marker in red. Scale bar represents 1μm.

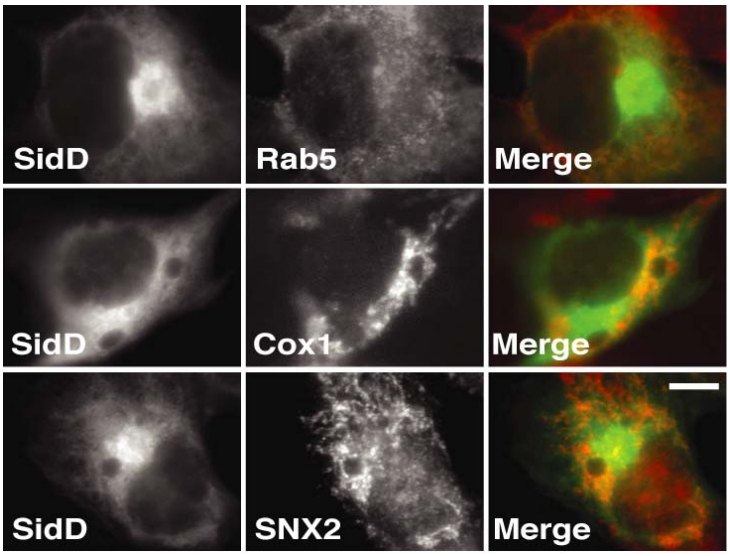

Fig. S10. GFP-SidD does not colocalize with the endosomal compartment, mitochondria, or recycling endosomes. Transiently transfected COS1 cells producing GFP-SidD (left panels) were fixed and stained for the indicated organelle marker (middle panels). The merged images (right panels) show SidD in green and the organelle marker in red. Scale bar represents 1μm. No co-localization of GFP-SidD with the marker proteins Rab5 (endosomal compartment), Cox1 (mitochondria), or sorting nexin 2 (SNX2; recycling endosomes) was detectable.

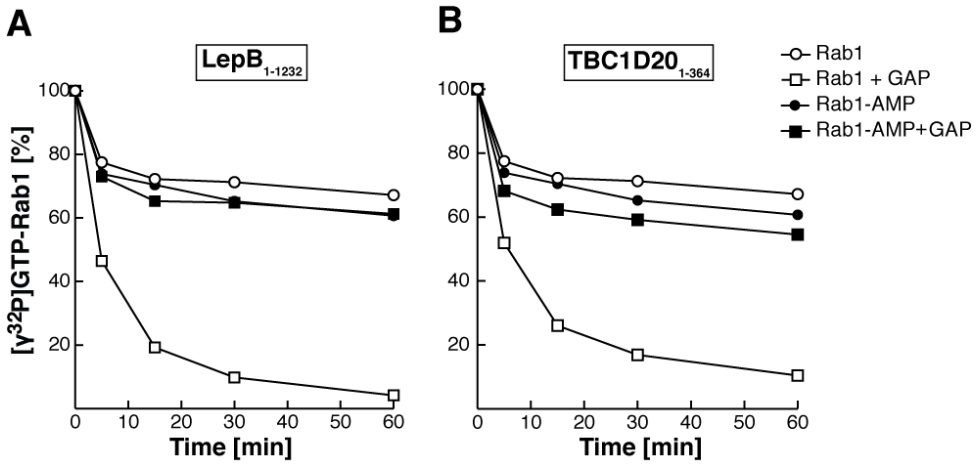

Fig. S11. AMPylated Rab1 cannot be inactivated by the GAPs LepB or TBC1D20. Rab1 or AMPylated Rab1 (1 μM each) loaded with [γ^{32}P]-GTP was incubated with 40 nM His-LepB$_{1-1232}$ (A) or His-TBC1D20$_{1-364}$ (B). Samples were taken at the indicated time points and [γ^{32}P]GTP hydrolysis was monitored by filter-binding assay.

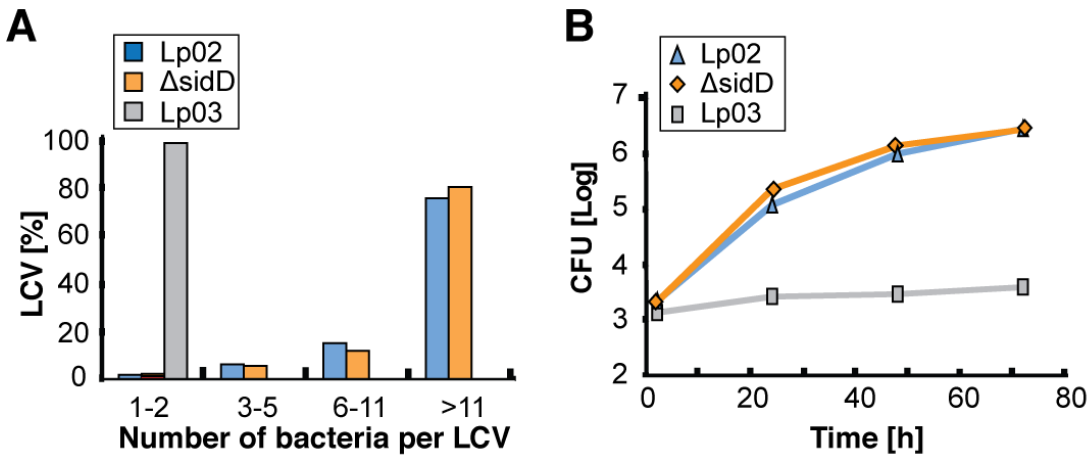

Fig. S12. Replication of *sidD* deletion mutants in U937 macrophages. (A) Efficiency of large replication vacuole formation of *L. pneumophila ΔsidD* mutants. U937 cells were challenged for 1 hour at a MOI of 5 with the indicated *L. pneumophila* strains (wild-type (Lp02), avirulent type IV-defective mutant (Lp03), *sidD* deletion mutant (*ΔsidD*)). Extracellular bacteria were removed, and infected cells were incubated for additional 13 hours at 37°C. The number of bacteria per vacuole 14 hours post infection was determined by fluorescence microscopy for at least 300 vacuoles per strain. (B) Three-day growth curve of *L. pneumophila* in U937 macrophages. U937 cells challenged with the indicated *L. pneumophila* strains at an MOI of 0.05 for 1 hour were washed to remove extracellular bacteria and incubated for an additional 71 hours to allow intracellular bacterial replication. Samples were taken at the indicated time points and colony-forming units (CFU) were determined by plating assay.

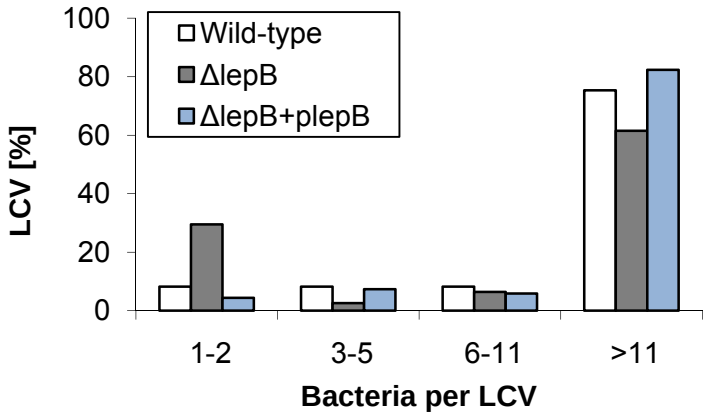

Fig. S13. *L. pneumophila lepB* deletion mutants are attenuated for intracellular replication. Intracellular replication in U937 macrophages was determined in an infectious center assay as described in Fig. S11. *ΔlepB* mutants failed to initiate intracellular replication three times more frequently than wild-type *L. pneumophila*.

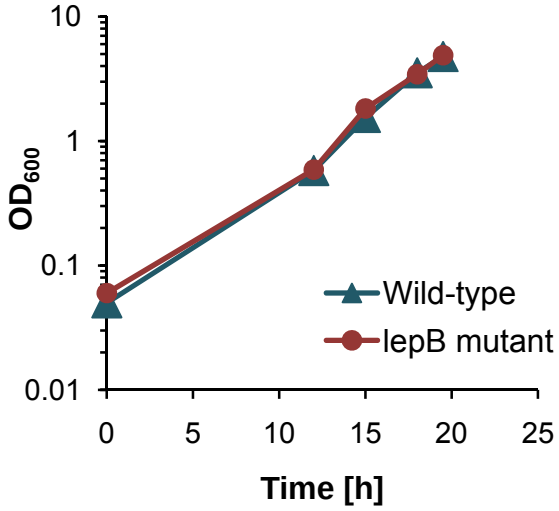

Fig. S14. *L. pneumophila lepB* deletion mutants replicate normally in liquid media. *L. pneumophila* wild-type and *ΔlepB* from an over night culture were diluted in fresh media to an OD_{600} of 0.05, and growth was monitored for 21 hours.

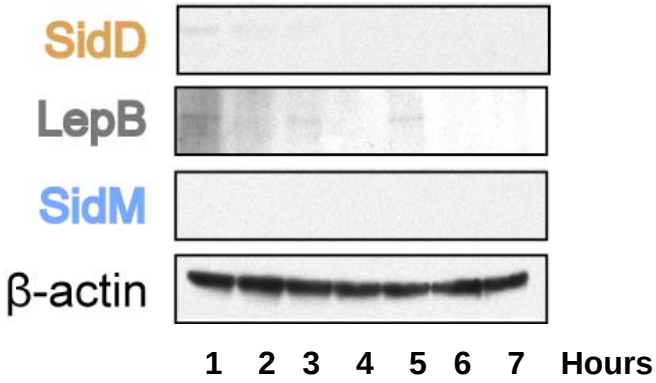

Fig. S15. Cytosol from host cells infected with T4SS-defective mutants does not contain translocated effector proteins. U937 cells were challenged with an *L. pneumophila* T4SS-defective mutant (Lp03), lysed at the indicated time points using 1% digitonin, and the digitonin-soluble fractions were analyzed by Western blot using antibody specific for the respective effector proteins or β-actin (loading control). This experiment confirms that 1% digitonin does not lyse intracellular bacteria but solubilizes only translocated effectors, as shown in Fig. 4E, F of the main text.

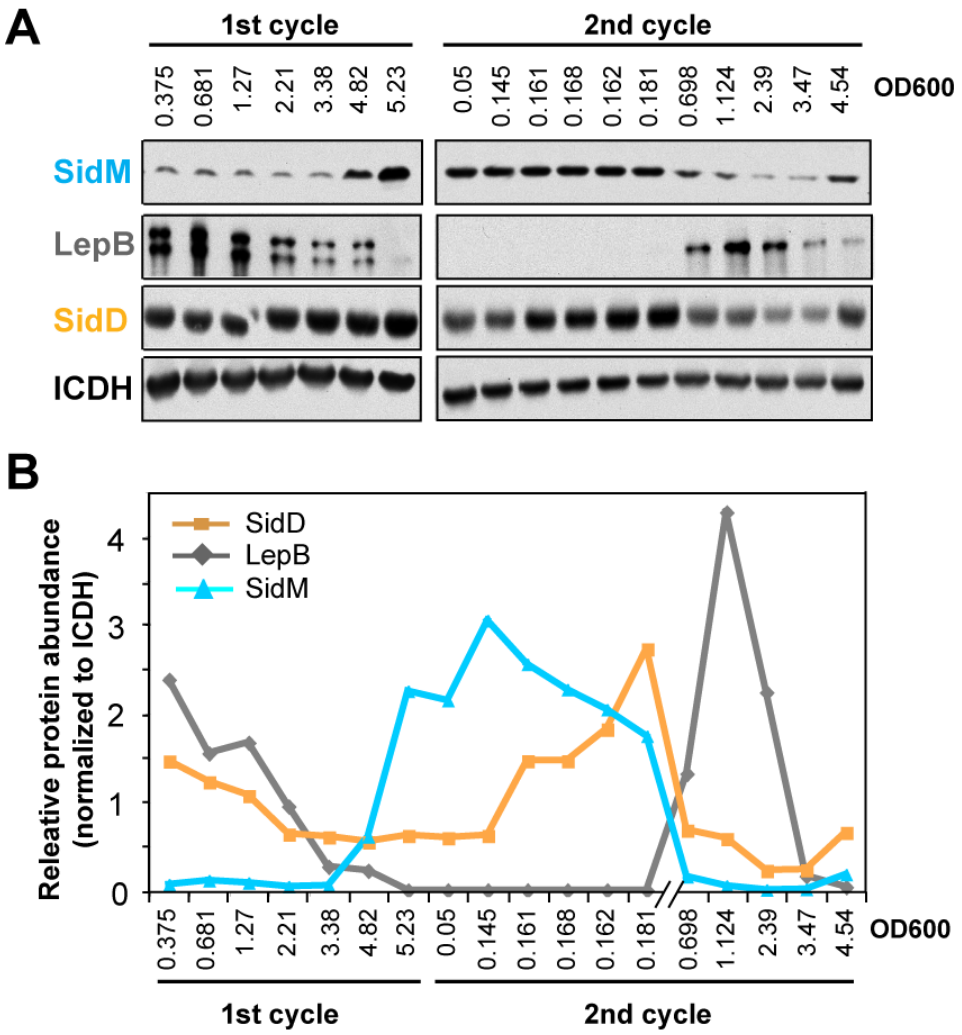

Fig. S16. Production of SidD, SidM and LepB during *L. pneumophila* growth in liquid culture. The biphasic intracellular life cycle of *L. pneumophila* can be simulated by growing the bacteria in liquid culture, with the exponential and stationary phase representing the replicative or transmissive (=virulent) intracellular form of *L. pneumophila*, respectively. In the experiment shown, a two-fold serial dilution (seven culture tubes total) of *L. pneumophila* was grown over night (1st cycle) in liquid media; bacteria from stationary growth phase (OD_{600} =5.23) were diluted into fresh media to an OD_{600} =0.05, and bacterial growth was monitored for an additional 24 hours (2nd cycle). Samples were taken in two-hour intervals (unless indicated otherwise //) and the OD_{600} was determined. Equal amounts of bacteria were resuspended in SDS-PAGE buffer, proteins were separated by SDS-PAGE and detected by Western blot using antibody against the indicated proteins. (B) Signal intensity shown in (A) was quantified by densitometry and normalized to the ICDH signal (loading control). Data are a representative of three independent experiments, and demonstrate that the level of SidD peaked prior to that of LepB, but later than SidM, which is consistent with a role of SidD in switching form an early period dominated by Rab1 recruitment to a later period of Rab1 removal during infection.

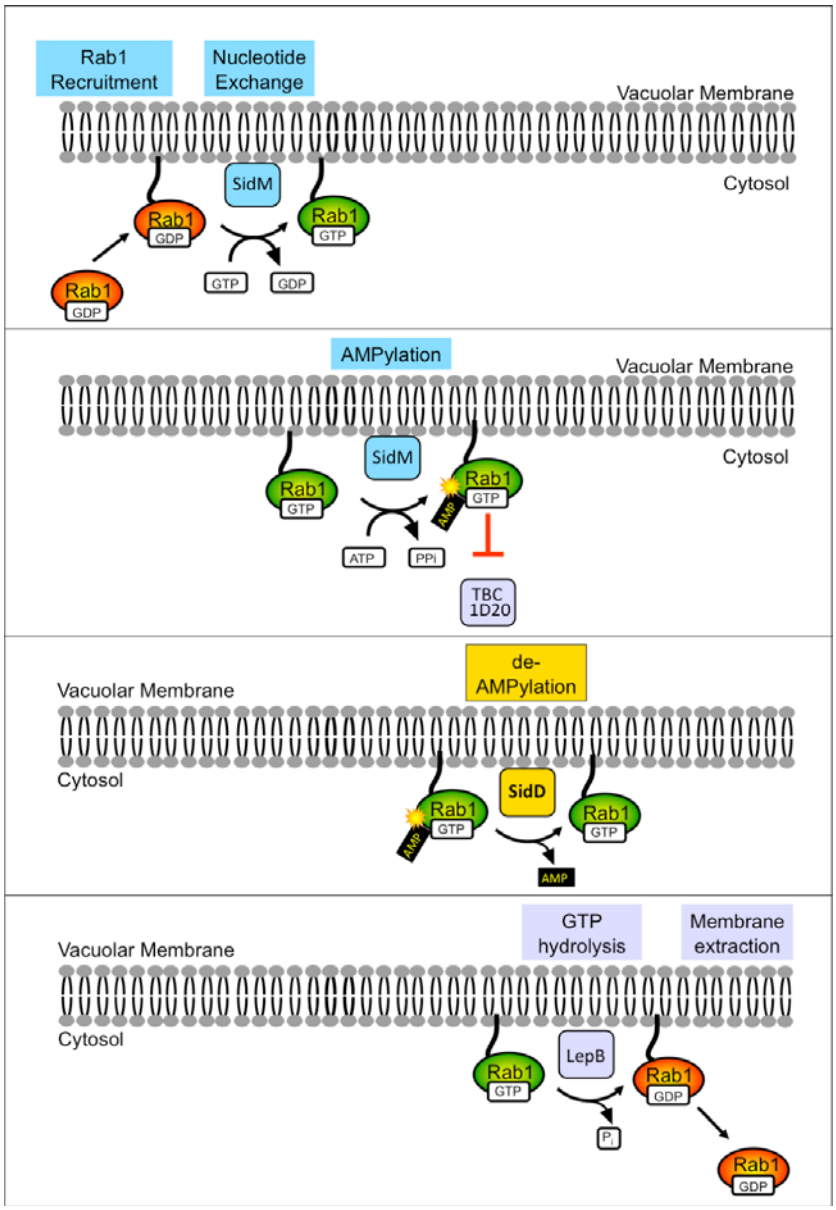

Fig. S17. Model of Rab1 modulation by *L. pneumophila* effector proteins. See main text for details.

SUPPORTING TABLES

Table S1. Plasmids used in this study. Oligonucleotides used for cloning are summarized in Supporting Table 2.

Name	Insert	Oligonucleotides	Source or reference
pGEX6p1-rab4a	Human rab4a	P17, P18	This study
pGEX6p1-rab6a	Human rab6a	P27, P28	This study
pGEX6p1-rab8a	Human rab8a	P19, P20	This study
pGEX6p1-rab14	Human rab14	P21, P22	This study
pGEX6p1-rab15	Human rab15	P23, P24	This study
pGEX6p1-rab35	Human rab35	P25, P26	This study
pQE80L-*sidD*	*L. pneumophila sidD* ORF	P8, P9	This study
pQE80L-TBC1D20$_{1-364}$	Human TBC1D20 (Thr364stop)	P1, P2	This study
pEGFP-*sidD*	*L. pneumophila sidD*	P8. P9	This study
pmCherry-*sidM*	*L. pneumophila sidM*	P7, P16	This study
pSR47s-*sidD*	*L. pneumophila sidD* flanking regions	P10, P11, P12, P13	This study
pQE80L-*lepB*	*L. pneumophila lepB*	P14, P15	This study
pQE80L-*lepB*$_{1-1232}$	*L. pneumophila lepB* (Trp1232stop)	P5, P6	This study
pGEX6p1-*sidM*	*L. pneumophila sidM*		(*6*)
pGEX6p1-rab1a	Human rab1a		(*6*)
pGEX6p1-rab2	Human rab2		(*6*)
pQE80-rab1a	Human rab1a	P29, P30	This study
pSR47s-*lepB*	*L. pneumophila lepB* flanking regions	P31, P32, P33, P34	This study
pJB908-*lepB*	*L. pneumophila lepB*	P35, P36	This study
pJB908-*sidD*	*L. pneumophila sidD*		This study

Table S2. Oligonucleotides used in this study. Capital letters indicate nucleotide restriction sites.

Number	Name	Sequence
P1	5'TBC_BglII	gatcAGATCTgccctccggagtgcgc
P2	3'TBC364stop	aaaGTCGACttagcggttggtccttggc
P3	5'lepB_BamHI	gatcGGATCCatgttaatttatcaaggtaa
P4	3'lepB_SalI	gatcGTCGACttatccataaactaatgtttc
P5	5'LepB1232stop	caacatcgtgaagaatAAAGATCTctgttagccaatgttac
P6	3'LepB1232stop	gtaacattggctaacagAGATCTTTattcttcacgatgttg
P7	5sidM_HindIII	atcAAGCTTCGttggtatattatgagatcattaag
P8	5sidD_BamHI	atcGGATCCttggtatattatgagatcattaag
P9	3sidD_SalI	atcGTCGACttaaatagtaagactcgagttag
P10	5sidD_up	atcGTCGACatatccgctgatgttaccc
P11	3sidD_up	atcGAATTCgacaatatccttaatgatctc
P12	5sidD_dn	atcGAATTCaaggctgcaactaactcgag
P13	3sidD_dn	atcGGATCCcatttgccagcacccattc
P14	5'lepB_BamHI	gatcGGATCCatgttaatttatcaaggtaa
P15	3'lepB_SalI	gatcGTCGACttatccataaactaatgtttc
P16	3sidM_SalI	atcGTCGACttaaatagtaagactcgagttag
P17	5Rab4a-BamHI	aaGGATCCtcgcagacggccatgtc
P18	3Rab4a-SalI	aaaGTCGACctaacaaccacactcctgag
P19	5Rab8a-BamHI	aaGGATCCgcgaagacctacgattacc
P20	3Rab8a-SalI	aaaaGTCGACtcacagaagaacacatcgga
P21	5Rab14-BamHI	aaGGATCCgcaactgcaccatacaactac
P22	3Rab14-SalI	aaaaGTCGACttaacagccacagccttctc
P23	5Rab15-BamHI	aaGGATCCgcgaagcagtacgatgtgc
P24	3Rab15-SalI	aaaaGTCGACtcagacgcgtgaatgactct
P25	5Rab35-BamHI	aaGGATCCgcccgggactacgaccac
P26	3Rab35-SalI	aaaaGTCGACttagcagcagcgtttctttcg
P27	5Rab6a'-BamHI	gatcGGATCCatgtccacgggcggag
P28	3Rab6a'-SalI	gatGTCGACttagcaggaacagcctc
P29	5'Rab1a-BamHI	atgGGATCCtccagcatgaatcccggaat
P30	3'Rab1a-SalI	atgGTCGACttagcagcaacctccacc
P31	5'up-lepB short	cgacgtcgacactacaaaaatgaa
P32	3' up-lepB	aaagaattcaactatttctttaccttg
P33	5' dn-lepB	gcggaattctccactcactccgaaac
P34	3'dn-lepB short	taaggagctcaaagccaatgcatt
P35	5'OE-lepB	aagtctagattatccataaactaatg
P36	3'OE-lepB	agagagctcatgttaatttatcaagg

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