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Supplemental Information

Regulation of Phosphoribosyl-Linked

Serine Ubiquitination by

Deubiquitinases DupA and DupB

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Supplemental Information

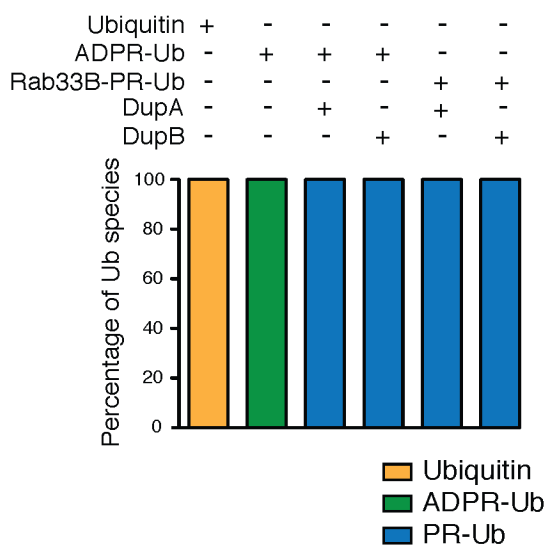
Regulation of phosphoribosyl-linked serine ubiquitination by deubiquitinases

DupA and DupB

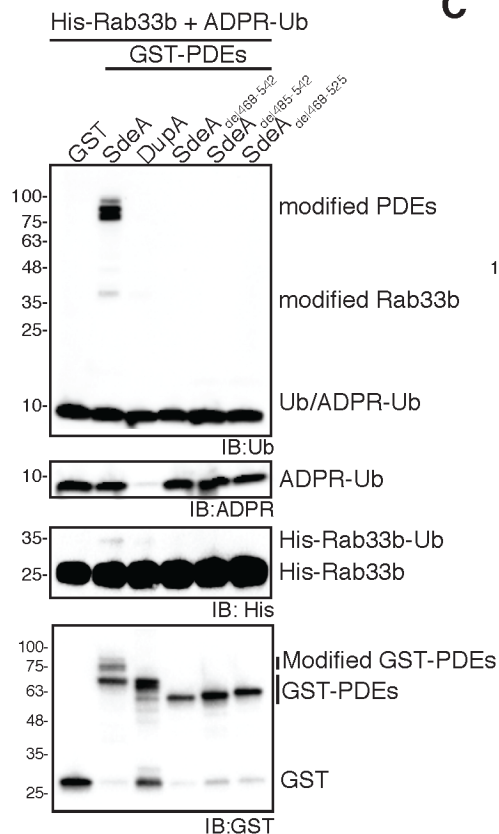
Shin et al.

Supplemental figure S1

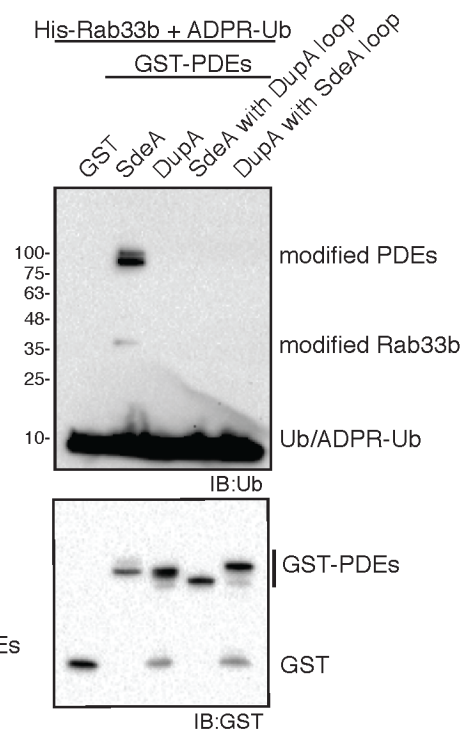
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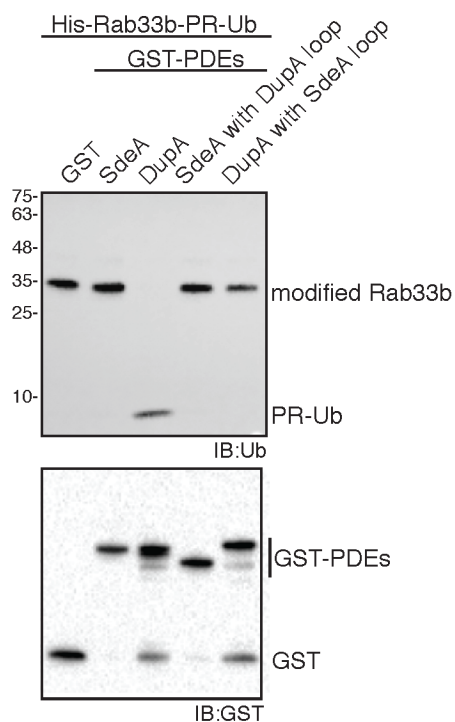
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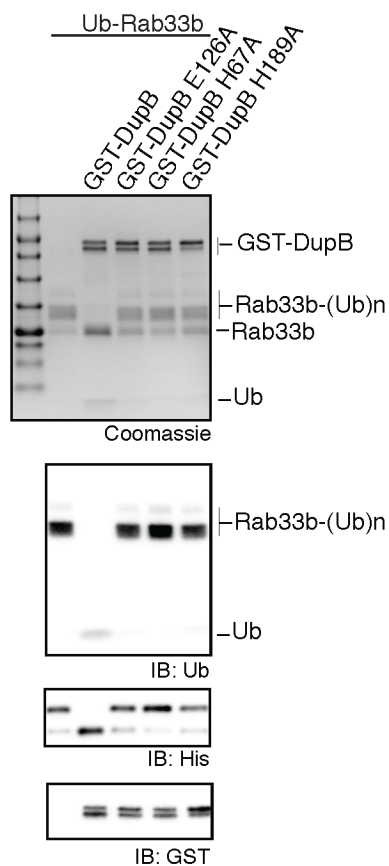
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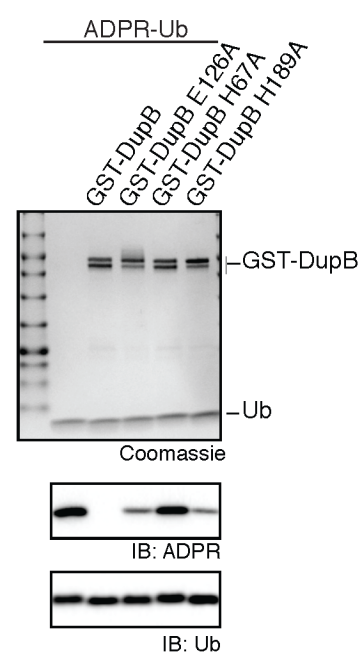
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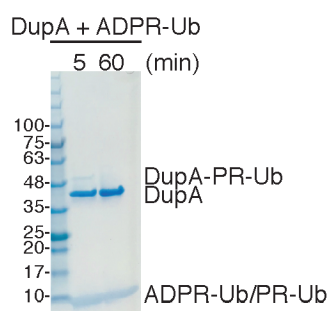
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F

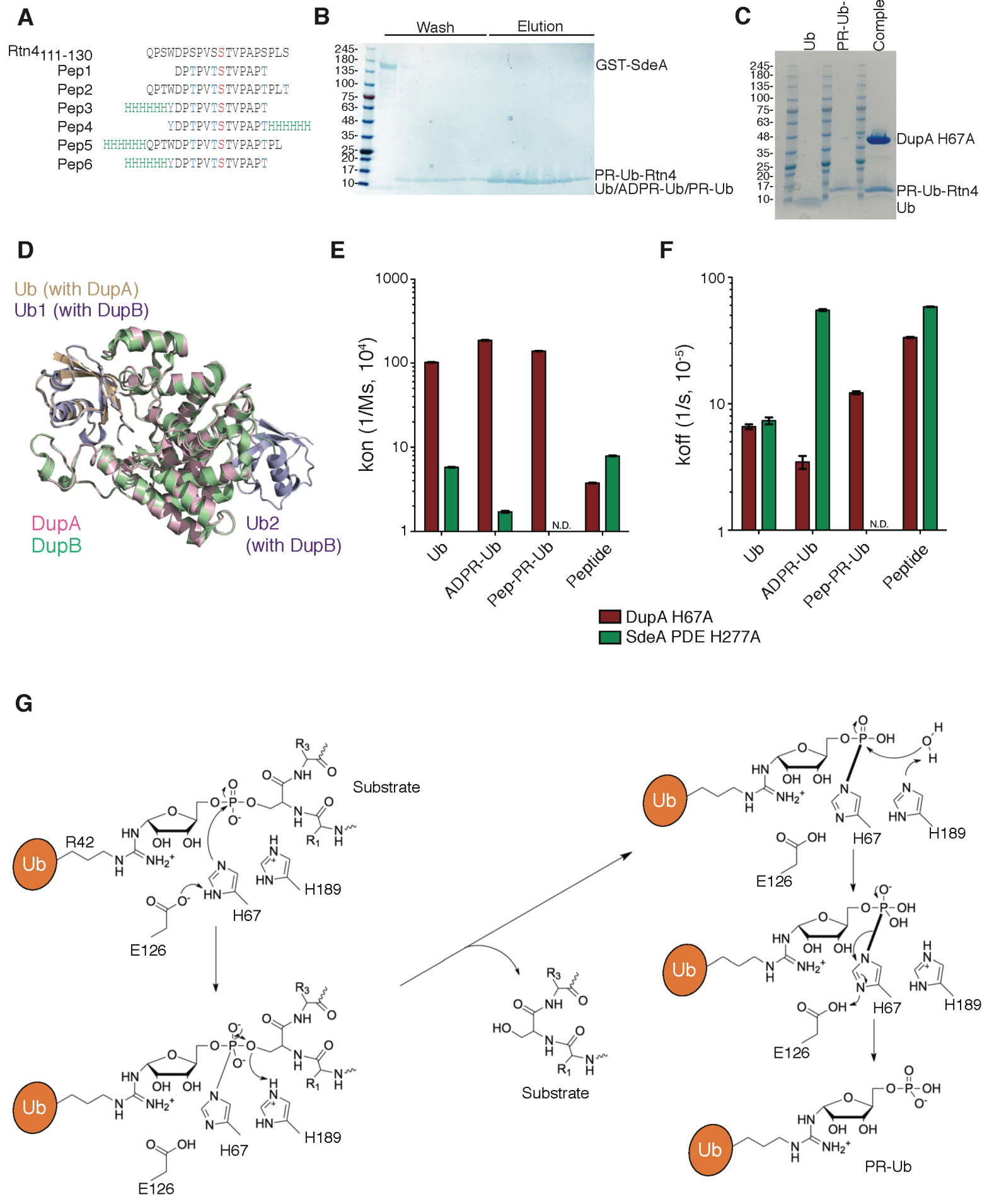


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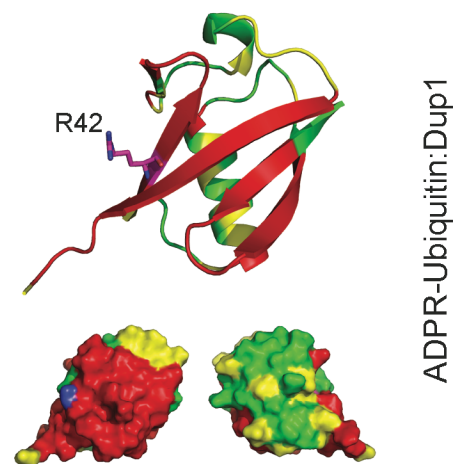
Supplemental figure S1. Control data related to Figure 1 and Figure 2. (A) PR-Deubiquitination assay of legionella lysates. PR-ubiquitinated-Rab33b was incubated with *Legionella* lysates as indicated. (B and C) PR-Ubiquitination assays of SdeA PDE loop deletion mutant, loop swapping mutant, respectively. (D) PR-Deubiquitination assay of DupA, SdeA PDE loop swapping mutant. (E and F) Deubiquitination assay of DupB wild-type and catalytically inactive mutants on PR-ubiquitinated Rab33b and ADPR-ubiquitin, respectively. (G) Time-course auto-PR-ubiquitination assay of DupA wild-type.

Supplemental figure S2



Supplemental figure S2. Synthesis of PR-ubiquitinated Rtn4 peptide and binding kinetics of DupA_{H67A} and SdeA PDE_{H277A} - related to Figure 3. (A) Original Rtn4 peptide was modified to have single serine residue for homogenous modification. (B) Purification of PR-ubiquitinated His-tagged Rtn4 peptide. (C) DupA H67A and PR-ubiquitinated Rtn4 complex mixture used for crystallization. (D) Superimposition of DupA:Ubiquitin complex structure with DupB (SdeD):Ubiquitin structure (PDB ID: 6B7P). (E and F) Association rate (k_{on}) and dissociation rate (k_{off}) of catalytically inactive DupA_{H67A} and SdeA PDE_{H277A} to ubiquitin species or peptide as indicated. Data are represented as mean \pm S.E.M. *S.E.M; standard error of mean. (G) Proposed catalytic mechanism of PR-ubiquitin cleavage by DUPs.

A



Supplemental figure S3. NMR titrations of the DupA_{H67A} into ¹⁵N-labeled Ub and ADPR-Ub - related to Figure 3.

(A) Representative sections of [¹⁵N, ¹H]-SOFAST-HMQC spectra for free ¹⁵N-labeled Ub (left), the Ub in presence of DupA (right), and overlay of both (middle). **(B)** The same experiments for ¹⁵N-labeled ADPR-Ub. Resonance assignments are shown where possible; the assignments for the Ub resonances at the final titration stage represent the most likely assignments based on the resonances of free Ub. While the Ub-DupA complex shows intermediate exchange dynamics with many resonances disappearing, the ADPR-Ub-DupA complex shows slow exchange. **(C)** CSP values observed for the ¹⁵N-labeled Ub resonances upon titration with non-labelled DupA (at a molar ratio of 1:3.2) and mapped on the Ub protein sequence. Yellow and red lines indicate the 1x and 2x standard deviations (SD) calculated from CSP values of all Ub HN resonances which can be traced over the titration experiment. CSP bars for residues with small (CSP < SD), intermediate (SD < CSP < 2xSD) or strong (2xSD < CSP) CSPs are marked in green, yellow and red, respectively. CSP bars for residues with disappearing resonances are set to 0.3 ppm and colored gray. **(D)** CSP values mapped on the Ub protein structure (PDB ID: 1UBQ) shown as a ribbon diagram (upper panel) and as protein surfaces (lower panel, showing two orientations differing by a 180° rotation around the Y axis). Residues with small (CSP < SD), intermediate (SD < CSP < 2xSD) or strong (2xSD < CSP) CSPs were marked in green, yellow and red, respectively. All residues with disappeared HN signal were also marked red. The R42 sidechain is shown as sticks in the ribbon diagram and marked blue on the surface representations. **(E)** CSP values observed for the ¹⁵N-labeled ADPR-Ub resonances upon titration with non-labelled DupA (at a molar ratio of 1:2) and mapped on the Ub protein sequence. Yellow and red lines indicate the 1x and 2x standard deviations (SD) calculated from CSP values of all Ub HN resonances which can be traced over the titration experiment. CSP bars for residues with small

($CSP < SD$), intermediate ($SD < CSP < 2xSD$) or strong ($2xSD < CSP$) CSPs are marked in green, yellow and red, respectively. CSP bars for residues which could not be assigned due to unambiguity are set to 0.4 ppm and colored gray. **(F)** CSP values mapped on the Ub protein structure (PDB ID 1UBQ) shown as a ribbon diagram (upper panel) and as protein surfaces (lower panel, showing two orientations differing by a 180° rotation around the Y axis). Residues with small ($CSP < SD$), intermediate ($SD < CSP < 2xSD$) or strong ($2xSD < CSP$) CSPs were marked in green, yellow and red, respectively. All residues with disappeared HN signal were also marked red. The R42 sidechain is shown as sticks in the ribbon diagram and marked blue on the surface representations.

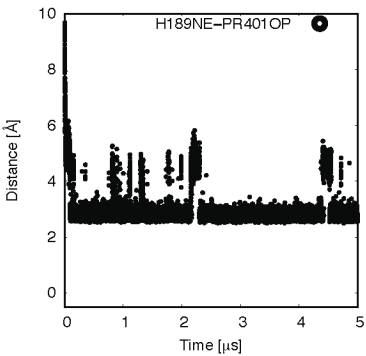
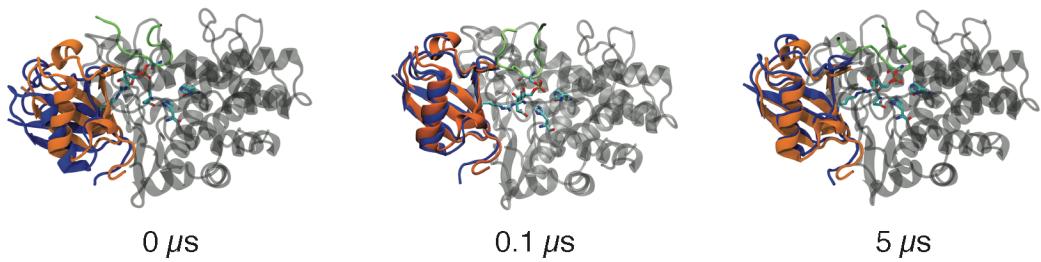
Supplemental figure S4

Ubiquitin (simulation)

Ubiquitin (DupA:Ub crystal structure)

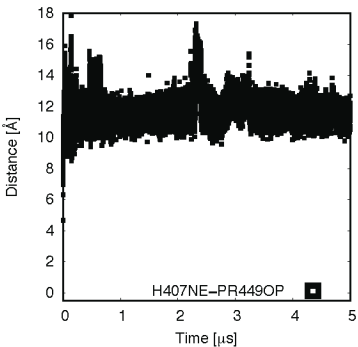
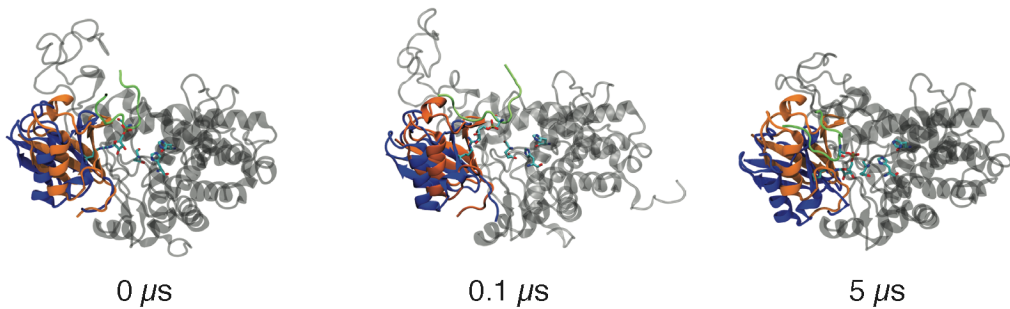
A

DupA with Ubiquitin (starting from SidE position)



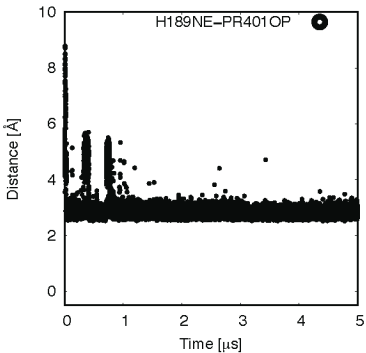
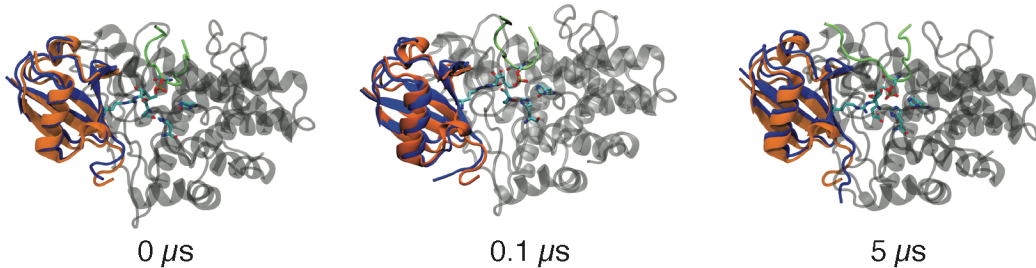
B

SdeA PDE with Ubiquitin (starting from SidE position)



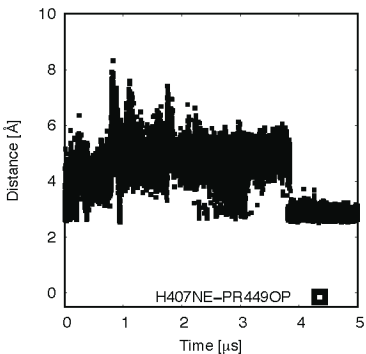
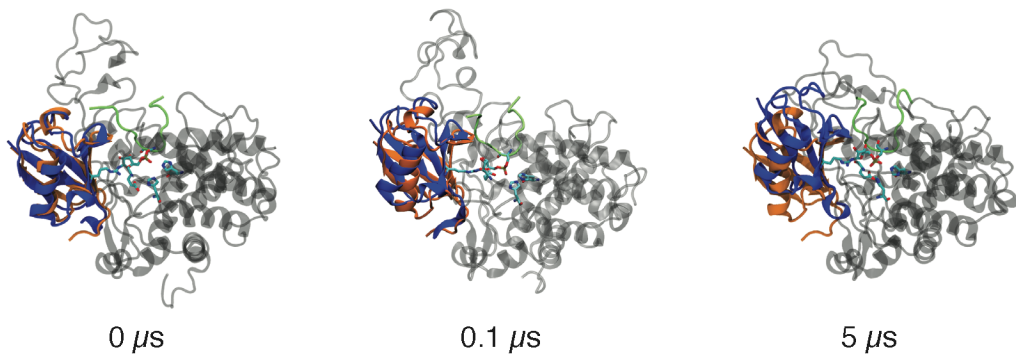
C

DupA with Ubiquitin (starting from Dup1 position)



D

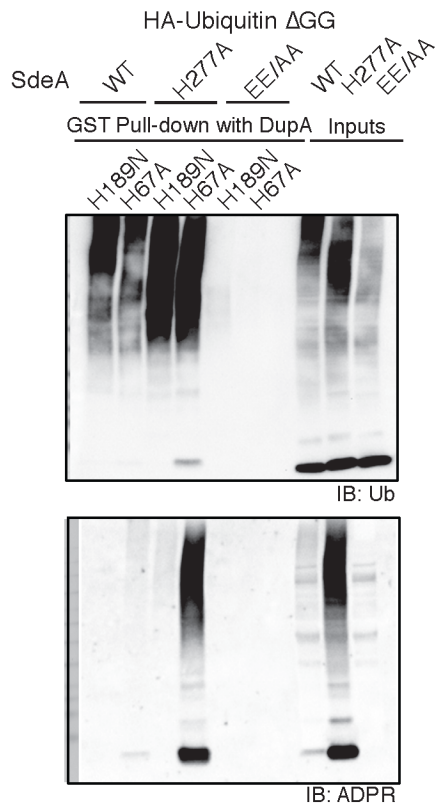
SdeA PDE with Ubiquitin (starting from DupA position)



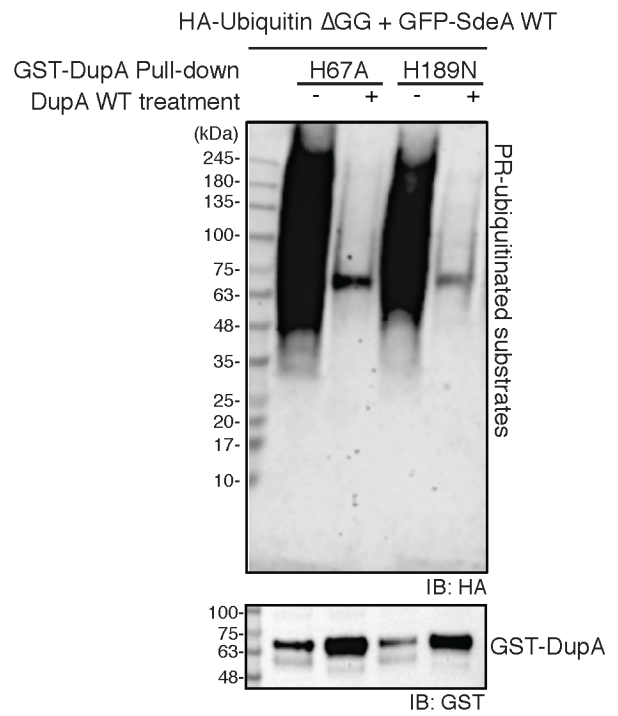
Supplemental figure S4. Molecular dynamics simulation of DupA and SdeA PDE with PR-ubiquitinated Rtn4 peptide - related to Figure 3. (A and B) MD simulation of PR-ubiquitinated-Rtn4 located in SidE ubiquitin position with DupA and SdeA PDE, respectively. (C and D) MD simulation of PR-ubiquitinated-Rtn4 located in DupA ubiquitin position with DupA and SdeA PDE, respectively. Subset graph shows distance between H189 of DupA or H407 of SdeA PDE and phosphate from PR-ubiquitinated Rtn4 peptide.

Supplemental figure S5

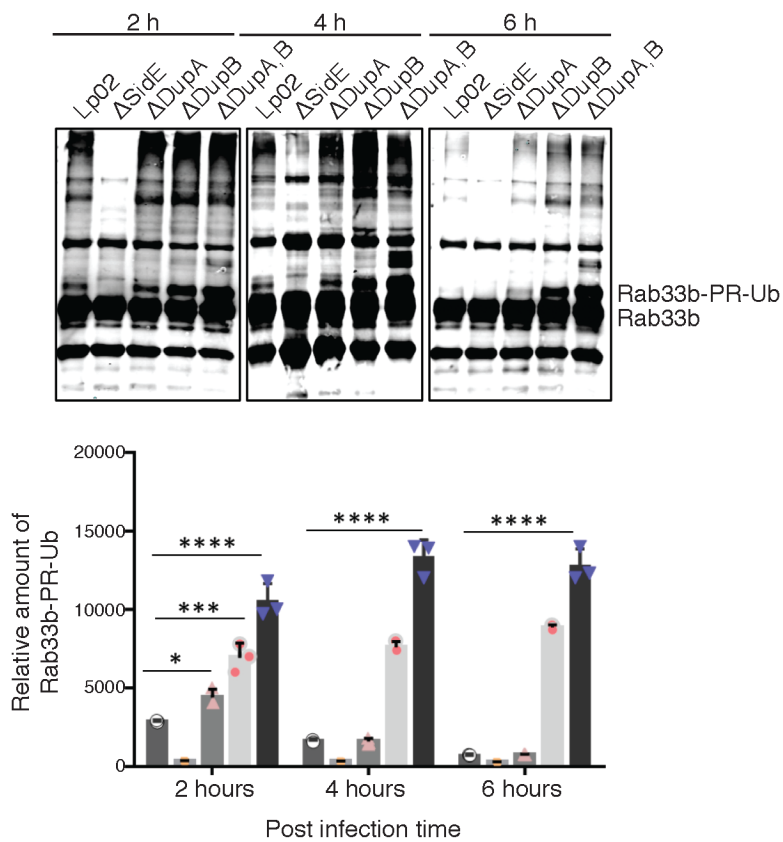
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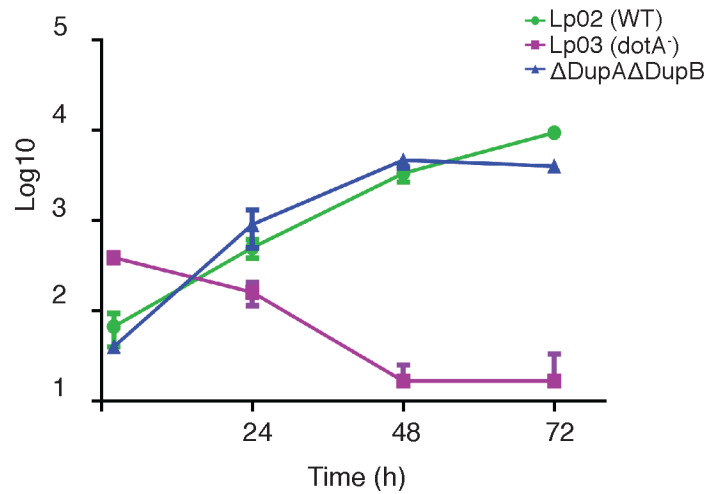
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C



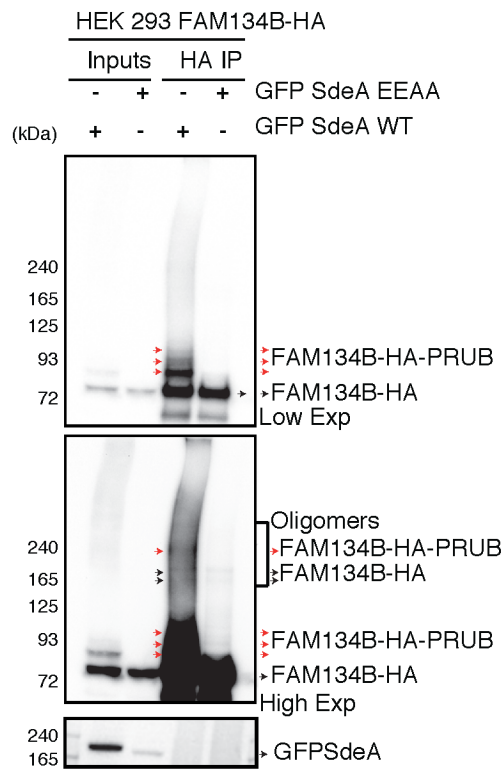
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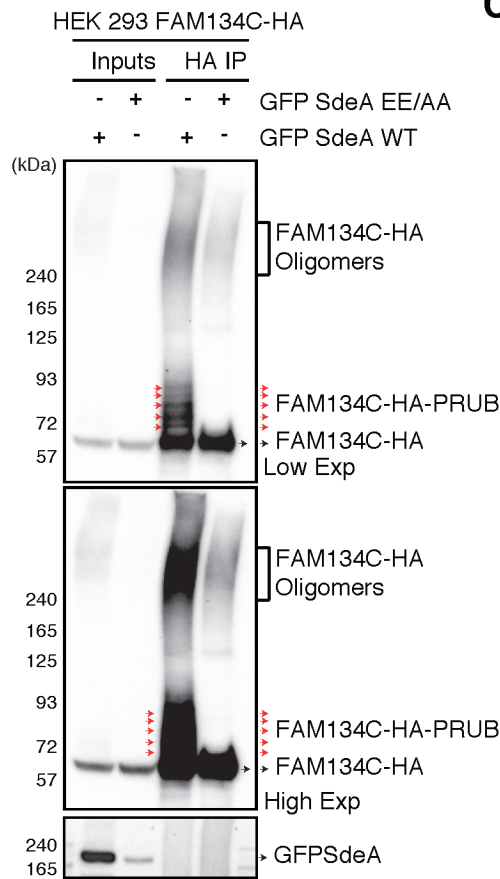
Supplemental figure S5. Enrichment of PR-ubiquitinated substrates from cells infected with Legionella strains - related to Figure 4. (A) Control Ubiquitin and ADPR blot for Figure 4A **(B)** Trapping endogenous PR-ubiquitinated substrates from cells transfected with SdeA and Ubiquitin Δ GG. Samples were further incubated with DupA WT to elute substrates. **(C)** PR-ubiquitinated Rab33b from cells infected with Legionella strains was monitored as indicated. **(D)** Mouse bone marrow-derived macrophages were infected with Legionella strains and the proliferation of the bacteria was monitored at the indicated time points. Data are represented as mean \pm SD.

Supplemental figure S6

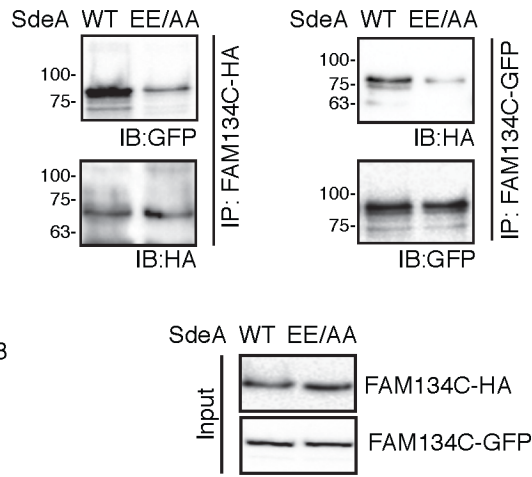
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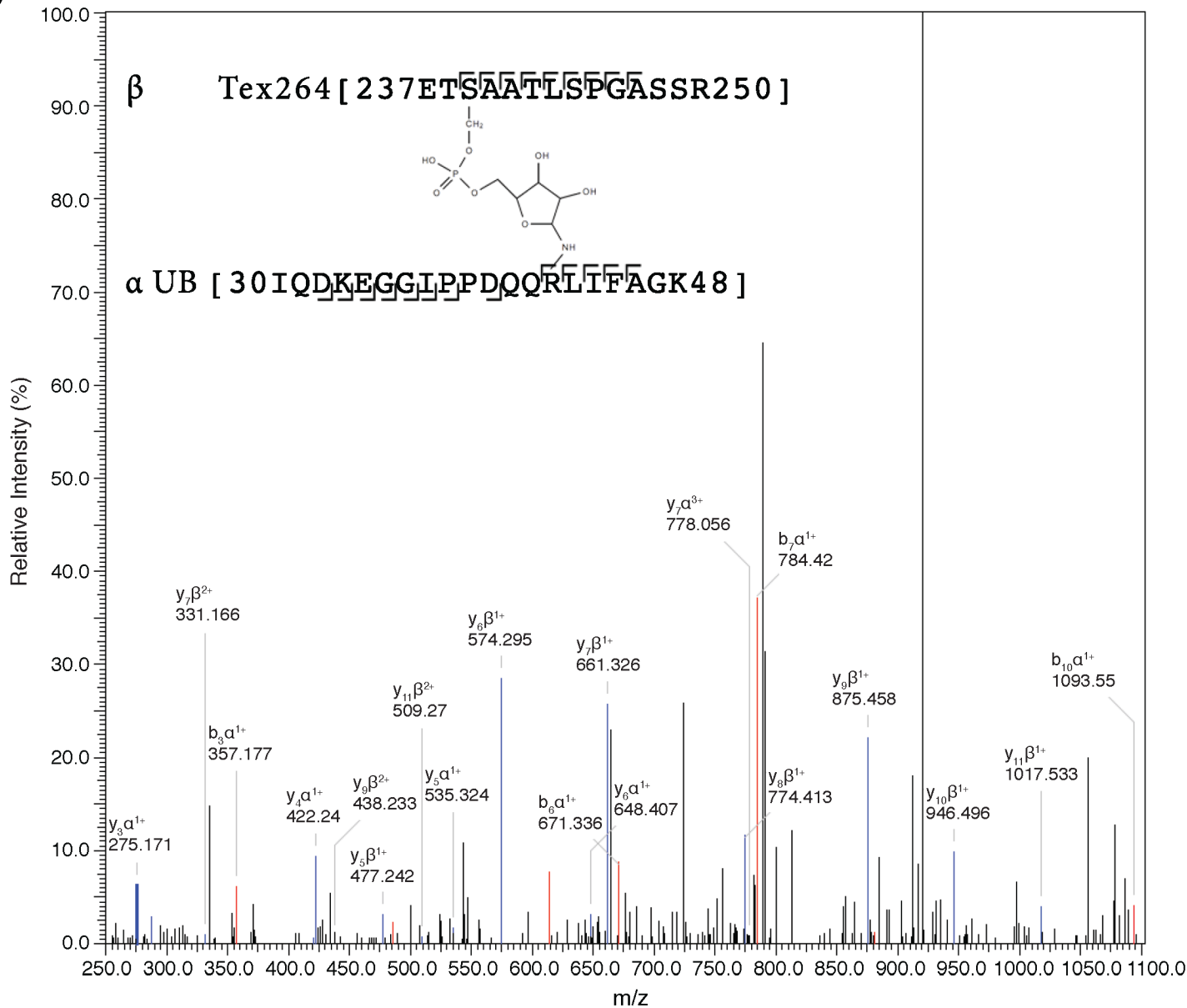
B



C



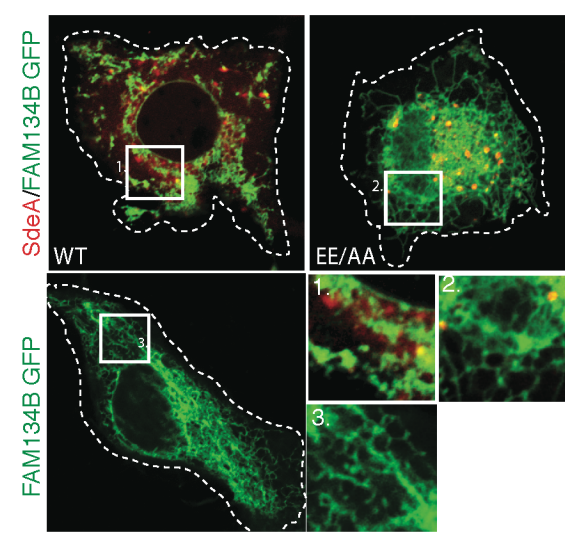
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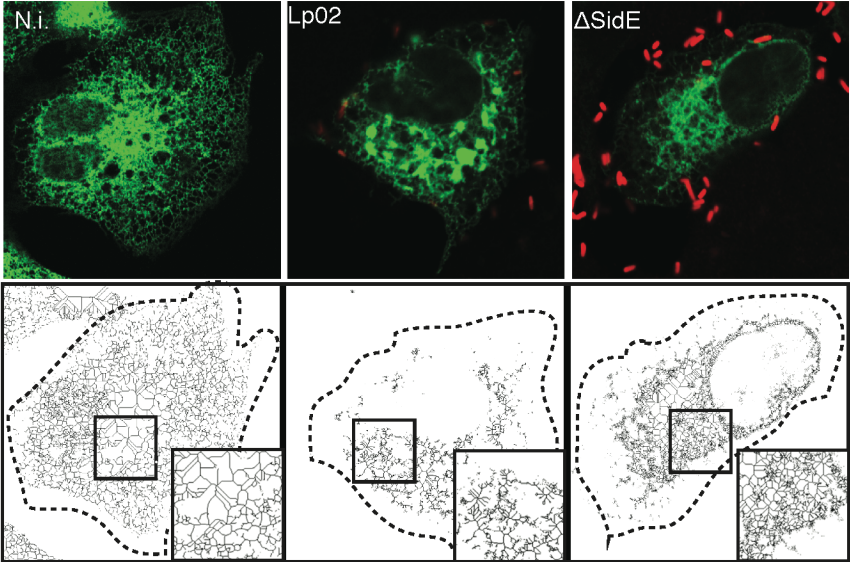
Supplemental figure S6-control data related to Figure 5 (A, B) HEK 293 HA-FAM134B or HA-FAM134C cells were transiently transfected with SdeA WT or EE/AA mutant, respectively. Soluble extracts were subjected to HA-IP and blotted against HA. Increased levels of the high molecular mass forms of HA FAM134B/C are immunodetected in SdeA WT transfected cells. **(C)** Lysates from cells co-transfected with HA-FAM134C, GFP-FAM134C and SdeA or SdeA(EE/AA) were used to immunoprecipitate HA-FAM134C and GFP-FAM134C. Oligomer formation was assessed by immunoblotting with GFP and HA respectively. **(D)** In vitro ubiquitinated Tex264 was digested with Trypsin and analyzed by LC-MS. PR-Ubiquitination could be mapped to Serine 239.

Supplemental figure S7

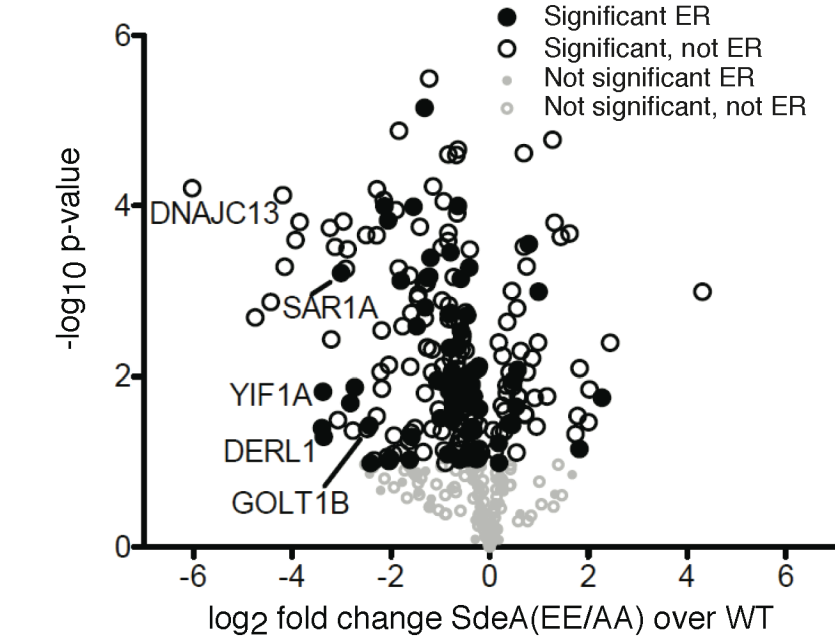
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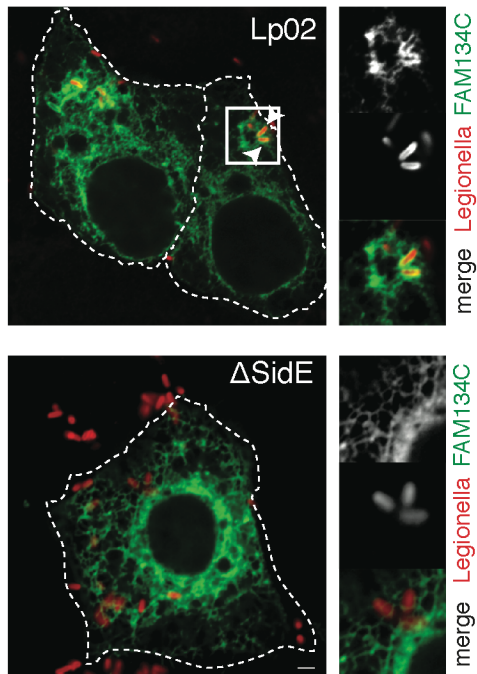
B



C



D



Supplemental figure S7-control data related to Figure 6

(A) Fragmentation of FAM134B marked ER in cells expressing SdeA. Graph represents 30 cells taken from 3 independent experiments. Error bars, represent standard deviation., $p < 0.05$. **(B)** Confocal images taken after 2h of infection were processed with ImageJ (converted to binary and skeletonized). More interconnected ER tubules are seen for control and delSdeA sets compared to cells with wild type *Legionella*. Error bars, represent standard deviation **(C)** Lysates from Hek293T cells expressing Lnp1-GFP, HA-SdeA or SdeA(EE/AA) were used for immunoprecipitation with GFP beads followed by mass spectrometric analysis to identify interactors of Lnp1 in both sets. **(D)** A549 cells were transfected with FAM134C-HA followed by infection with bacteria. 2 hours post infection, cells were fixed and immuno-stained using antibodies against *Legionella* and HA. Graph represents 30 cells taken from 3 independent experiments. Error bars, represent standard deviation, $p < 0.05$

Supplemental figure S8

Lpg1496	1	-----NDYLLSANGSKAIDFVWSNFKLKHYPNFKLKQPDANVKAWEKHADWEHWSNMGGQDW-SLNTF-SGLVPRPNHGIAHTLRV	78
Lpg2523	1	-----QSFFESSRNIGVIDKLHKDYLSKPYDQSEKGGD-----PNGGDW-TLNCGNKTLHRPNHGLSHTLRG	62
DupA	1	-----MPIILDPVLEKVAEYVYQERLSKPYTEVGPEWEYNHKTPTATATG-----TGHNLRFIITIDQRLHRPIHGLAHTMRT	75
DupB	1	-----MPIILDSVLEVAEYVYKTRLSQPYTEVGSEWEYNKNPTATFAKG-----DGHNLRQYITIDGKQLHRPIHGLAHTMRT	75
SdeB	1	QVAPKYSLDSKLLLENDVLDITIKYVHKEYLGKPYPGPLKNPKAP-----EE-----G-----R-----LPPNEGPDGRPHGLAHTVRT	69
SdeC	1	QVAPKHSLSKLLLENDVLDITIKYVHKEYLGKPYPGPLKNPKAP-----EE-----G-----R-----LPPNEGPDGRPHGLAHTVRT	69
SdeA	1	QDKSVPVWNGFSLYTDITVKAQAAYQYDNYLGKPYTGVSVEAPAN-----FG-----G-----R-----MV-----YRQHHLGSLHTLRT	64
SidE	1	QEVSVPTWNGFSLYTDITRNAARYAYDNYLGKPYTGVEATPVN-----FG-----G-----C-----MV-----YRQHHLGSLHTLRT	64
Lpg1496	79	AQLVPVIAEFLKAY-----SGDP-----KFKQLTQKEIQKAQYMMFLFSVIGRENDMSWTDANHYQAFKEAYNGQYSKHIYATFKEINAQK	158
Lpg2523	63	AALVPEVLYSYATH-----STDKKTRDMVQNI SKDDIKRMQVAMLFSSVGRKSELGFADNPGVYGGYRRASAD-----LFEEYA--	136
DupA	76	LFYSQLMYEAAKRQ-----PHPHRCADGRTIADLSVQDLKKLNIAQLFFVAGRESEASYG-----DA-----YHRYHLYGAK	142
DupB	76	LMYSQLMYCSKKQ-----PSPHVCQDGRITADLSVQDLKKLNIAQLFFVAGRESEASYG-----DA-----YHRYHLYGAK	142
SdeB	70	MACAEVMI EEARKAQLRGETLGKAKNGQTLADVTPEELKKILIAQAFFVVGRODDERSGYDDVHKRNF-----YAEYHEKSEQ	146
SdeC	70	MACAEVMI EEARKAQLRGETLGKAKNGQTLADVTPEELKKILIAQAFFVVGRODDERSGYDDVHKRNF-----YAEYHEKSEQ	146
SdeA	65	MAYAEELIVEEARKAKLRGETLGKFKDGRITADVTPEELKKIMIAQAFFVAGRDDEASDA-----KN-----YQKYHEQSRD	135
SidE	65	MAYAEELIVEEARKAKLRGETLKTADFGRITADVTPEELKKIMIAQAFFVVGRODDERS-S-----KN-----YEKYHEQSRD	134
Lpg1496	159	GFLNHVVTNKSLIPSLFSDESELQYWAEA-LDTGKPGISSA-----SGILMALAHDLDMRCYDKGKFNLSLKMK-----DLVAR	232
Lpg2523	137	-----KTHYKDLFTSKECIAYWKRLVLDYGNPGFHPDVNKPBEQAI IAKIMRCHCLDLLRCYSKEEFTKKVTK-----PLQDD	209
DupA	143	QFEAYARKHLTH-----LF-SKEIVLYSRCIEDRIGDRFDET-----AEGYLIHLSHMDLMRCKSPVEVFIGHSRGVSGIVPTLIQL	220
DupB	143	QFEAYARKHLTH-----LF-SKEIVLYSRCIEDRIGDRFDET-----AEGYLIHLSHMDLMRCKSPVEVFIGHSRGVSGIVPTLIHL	220
SdeB	147	AFRKYVEDNK--LIGKIFKDKQKVDYFAAII LDKN-HEWDAT-----PAHILINQGHMVDLMRTKAPAEVALERTY-----NTLKGT	220
SdeC	147	AFRKYVEDNK--LIGKIFKDKQKVDYFAAII LDKN-HEWDAT-----PAHILINQGHMVDLMRTKAPAEVALERTY-----NTLKGT	220
SdeA	136	AFLKYVKDNESTLIPDVFKDQEDVNFYARVIEDKS-HDVEST-----PAHVLINQGHMVDLVRVVKQPPESFLQRYF-----SSMQRW	211
SidE	135	AFLKYVEENKSTLIPDVFKDEKDKVFXADVIEDK-HKWDAS-----PAHVLINQGHMVDLVRVVKQPPESYLEYF-----SQLQFW	210
Lpg1496	233	LGNGEDAACKLADYAHDLIVATGDRCMGYGVTTQDQNY---SL-----F-----GKCSLDPNECKLQQLQSIPKPEET	295
Lpg2523	210	LGV--DAANNLVNYSKTLLEKTGDRILGTKS---YNL---QE-----H-----WLNNTDVSHCINTLNSALQHA--	265
DupA	221	FGR--EDGLDIMHYARSLFAATGEAVPYISSSEW-PHLGIESDRVERALKIVGSLEVEGQEAQAKKTAQAQFSVDGCGYALVKIDTPDW	307
DupB	221	FGK--QDGLDIMHYARGLFAATGEAVPYISSSEW-PHLGVDLSRVQRALSIVGDIVNVPQGEADSKKTAQAQFSVDGCGYSALTSVPTPSW	307
SdeB	221	VGS--KSAEVVLKAHRDFFATGAVVPLVNPEAIDDP---SR--GGPYE--NPYSGEKFVIVDDK-----VPASKKD-----LPKAV	288
SdeC	221	VGS--KSAEVVLKAHRDFFATGAVVPLVNPEAIDDP---SR--GGPYE--NPYSGEKFVIVDDK-----VPASKKD-----LPKAV	288
SdeA	212	IGS--QATEAVFGIQRQFFHATYEVVAGFDSNKEPHLVVSG--LGRYV--IGEDGQPIREAPKK-----GQKEG-----DLKVF	280
SidE	211	IGS--TATEAVFATQRQFFHATYEAVALGDSNKEPHLVVDG--LGRYV--IGQDGNPIREESD-----EDEEESG-----ELKFF	281
Lpg1496	296	LANQYGI-----DMWKLL-----	302
Lpg2523	266	PHQQYVVKHHEEESLDS-----	288
DupA	308	-HQVKEKED--Y--DVD-----EVIALPPQITIREEPKTNESFLLSLL-RHVFWCCPSNOKRDDENTEVLEKKAQ-----	372
DupB	308	-EKELKEID--DEKIDVKEVDDREIEKEHEIVVPSQATTPLSTV-KTDSFVEKLLKPFMIWKKPEVQTTOTTEKT-NK-----	381
SdeB	289	-NRDYKLLKD-----NE-----RFLTIKYYAFPDVQQTYPGYKTRLEASS-----YY	329
SdeC	289	-NRDYKLLKD-----NE-----RFLTIKYYAFPDVQQTYPGYKTRLEASS-----YY	329
SdeA	281	-PQTYKLKE-----NE-----RLMRVDEFLKLPEIQNTFPGSGKHLQGGMPGMNEMDYW	328
SidE	282	-SQKKKLEE-----NQ-----RYMRVDEYLLKLDVQKRFPAGKKLQGGPLGLKEYQYL	329
Lpg1496			
Lpg2523			
DupA	373	-----L-----	373
DupB	382	-----P-----	382
SdeB	330	--FPTPFAGECEQNPAKCLGAIQKARSKLQTDAIKNGFQSSSEK	371
SdeC	330	--FPTPFAGECEHDPKCLGAIQKARSKLQTDAIKNGFQSSSDK	371
SdeA	329	NRLNSLNRRARCENDVDFCLKQLQTAHDKAKIEPIKQAFQSSSKG	372
SidE	330	QRLNSLNRRARCENDVDFCLKQLQTAHHQTKITPIKRAFQSSSEK	373

Supplemental figure S8. Multiple sequence alignment of Legionella PDEs related to figure 1. Sequences of eight PDE domains identified from pattern search were aligned using Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>).

Table S1 |Data collection and refinement statistics – related to figure 2 and 3

	DupA₄₋₃₄₅	DupA₄₋₃₄₅:Ubiquitin
Data Collection		
Space group	P 61 2 2	C 1 2 1
Cell dimensions		
a, b, c (Å)	87.259 87.259 612.618	116.33 ,67.14, 182.75
α , β , γ (°)	90, 90, 120	90, 90, 90
R_{merge}	0.1309 (2.371)	0.095 (0.484)
R_{pim}	0.02865 (0.5073)	0.065 (0.351)
$CC_{1/2}$	0.999 (0.452)	0.995 (0.672)
CC^*	1 (0.789)	0.999 (0.897)
$I / \sigma I$	19.11 (1.24)	7.28 (1.39)
Completeness	99.88 (99.22)	99.86 (99.96)
Redundancy	21.7 (22.1)	2.6 (2.4)
Refinement		
Resolution (Å)	47.59 – 2.315 (2.398 -2.315)	49.07 – 2.21 (2.289 – 2.21)
No. reflections	62183 (6017)	65182 (7030)
$R_{\text{work}}/R_{\text{free}}$	0.2174 /0.2475	0.2088 / 0.2888
No. atoms	7659	9240
Macromolecules	7492	9240
Solvent	167	0
B-factors		
Macromolecules	63.10	46.95
Solvent	54.65	-
R.m.s deviations		
Bond lengths (Å)	0.008	0.016
Bond angles (°)	0.94	2.13