Fig. S1

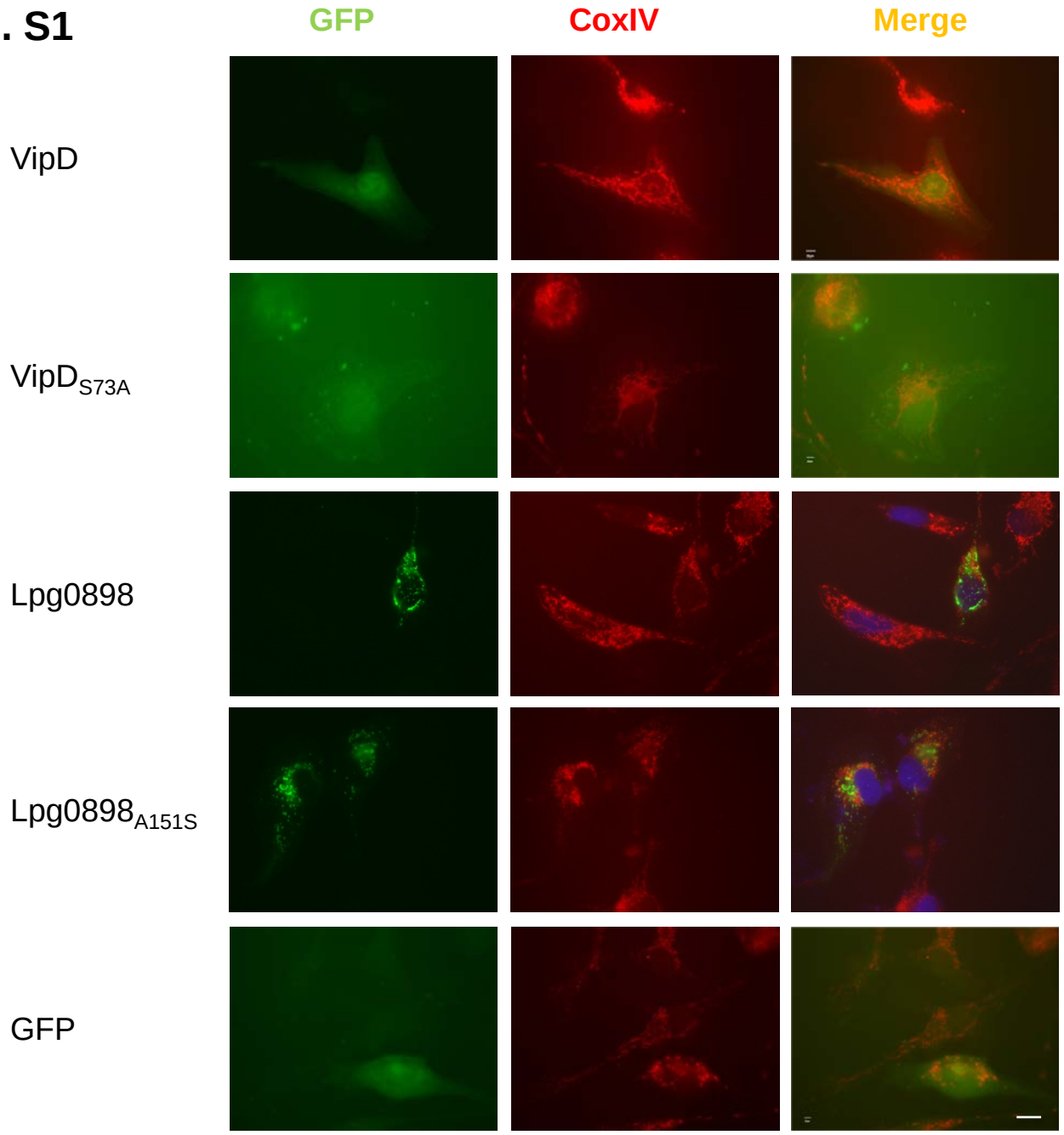


Fig. S2

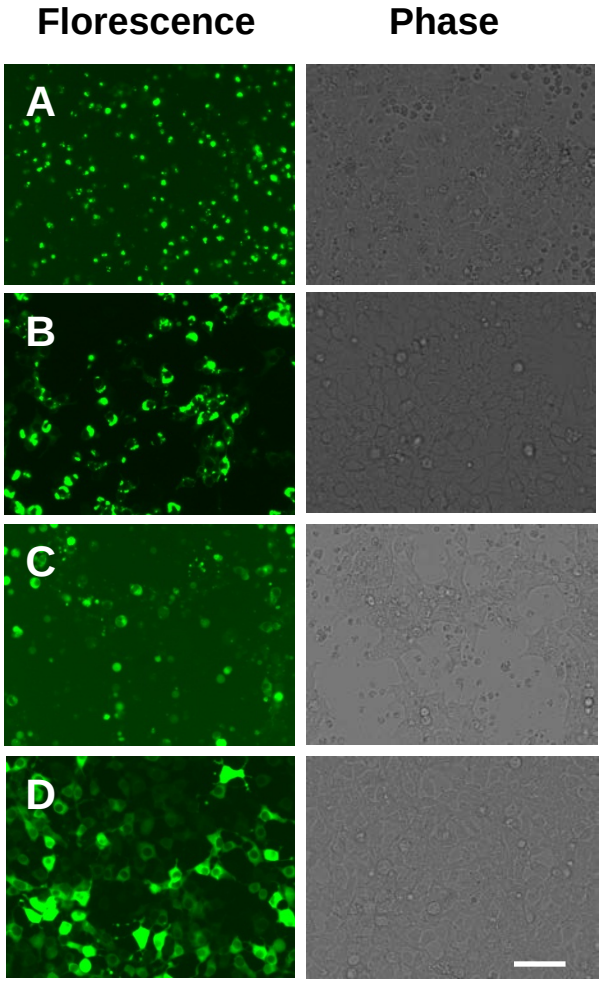


Fig. S3

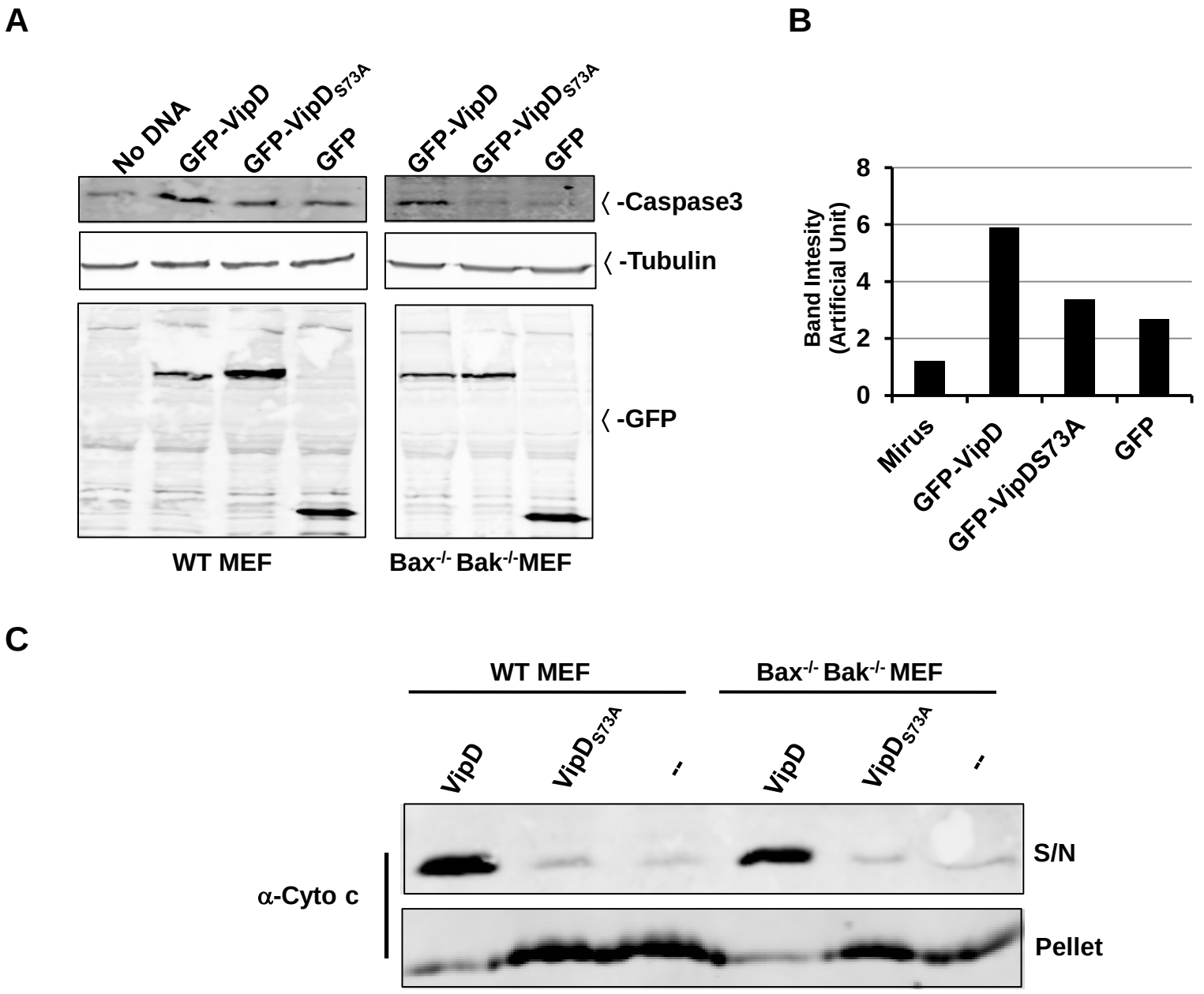

A
No DNA
GFP-VipD
GFP-VipDS73A
GFP
GFP-VipD
GFP-VipDS73A
GFP
〈 -Caspase3
〈 -Tubulin
〈 -GFP
WT MEF
Bax-/- Bak-/- MEF
B
Band Intesity (Artificial Unit)
8
6
4
2
0
Mirus
GFP-VipD
GFP-VipDS73A
GFP
C
WT MEF
Bax-/- Bak-/- MEF
VipD
VipDS73A
--
VipD
VipDS73A
--
α-Cyto c
S/N
Pellet

Fig. S4

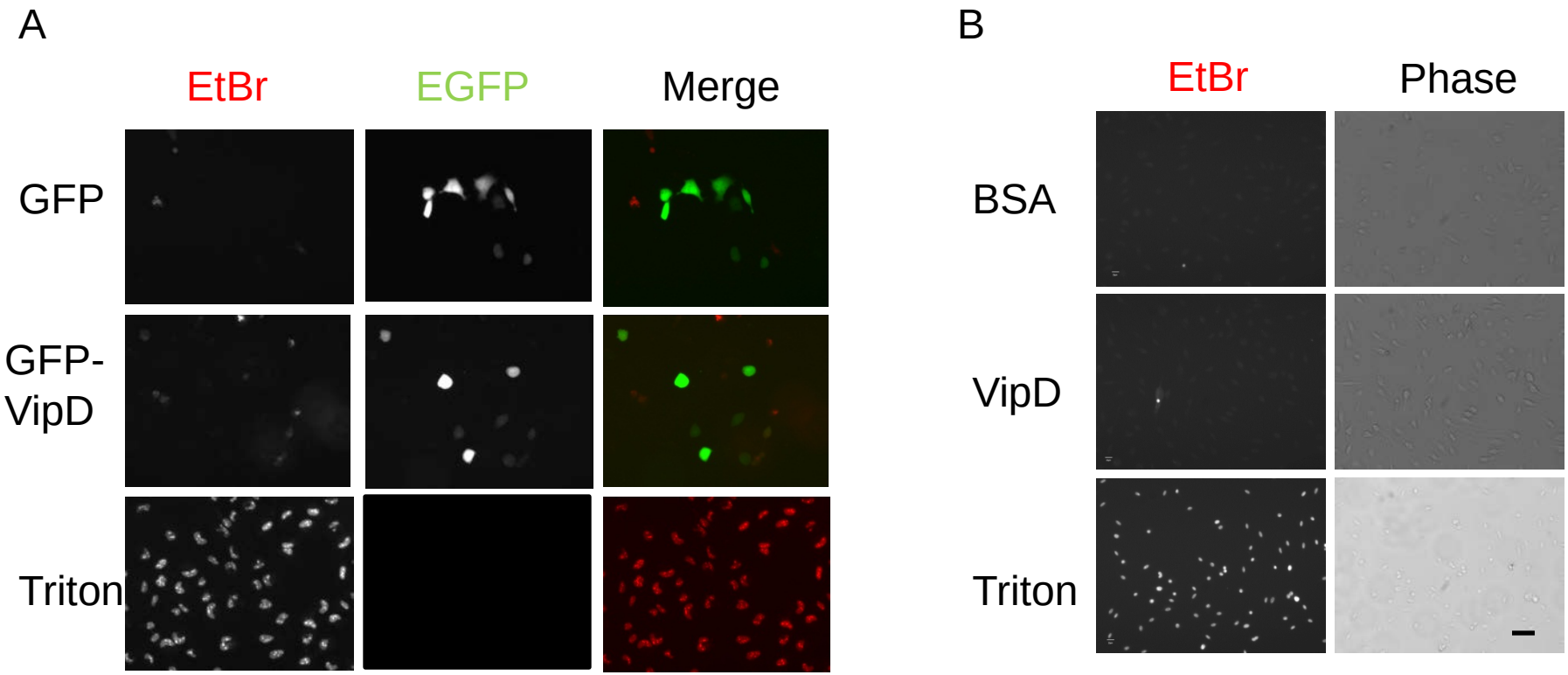
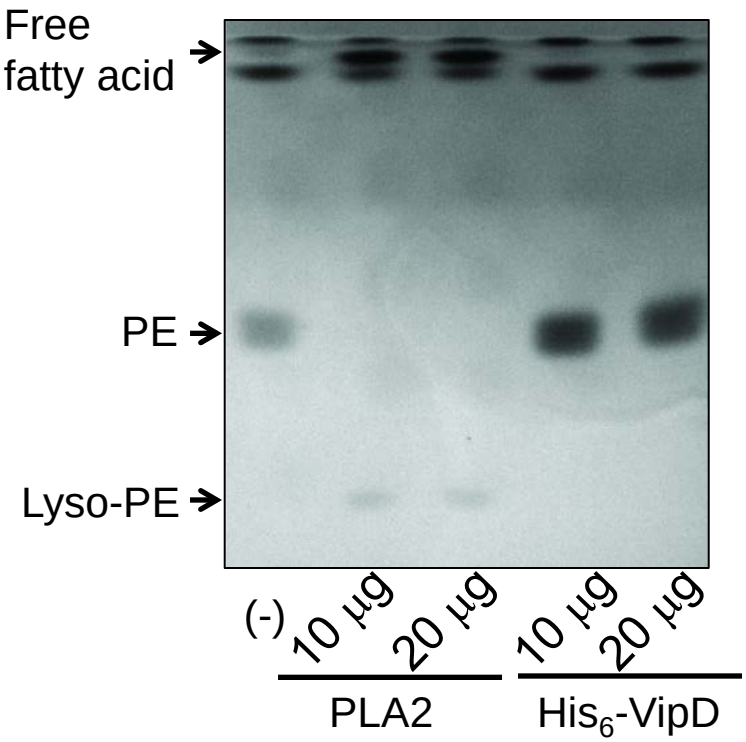

A
EtBr
EGFP
Merge
GFP
GFP-
VipD
Triton
B
EtBr
Phase
BSA
VipD
Triton

Fig. S5

A


Free
fatty acid
PE
Lyso-PE
(-)
10 μg
20 μg
10 μg
20 μg
PLA2
His6-VipD

B

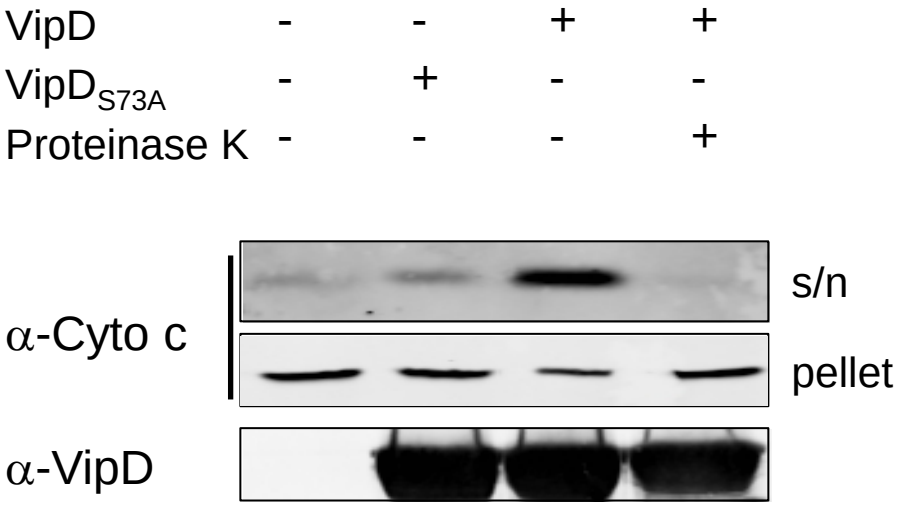

VipD - - + +
VipDS73A - + - -
Proteinase K - - - +
α-Cyto c
s/n
pellet
α-VipD

Fig. S6

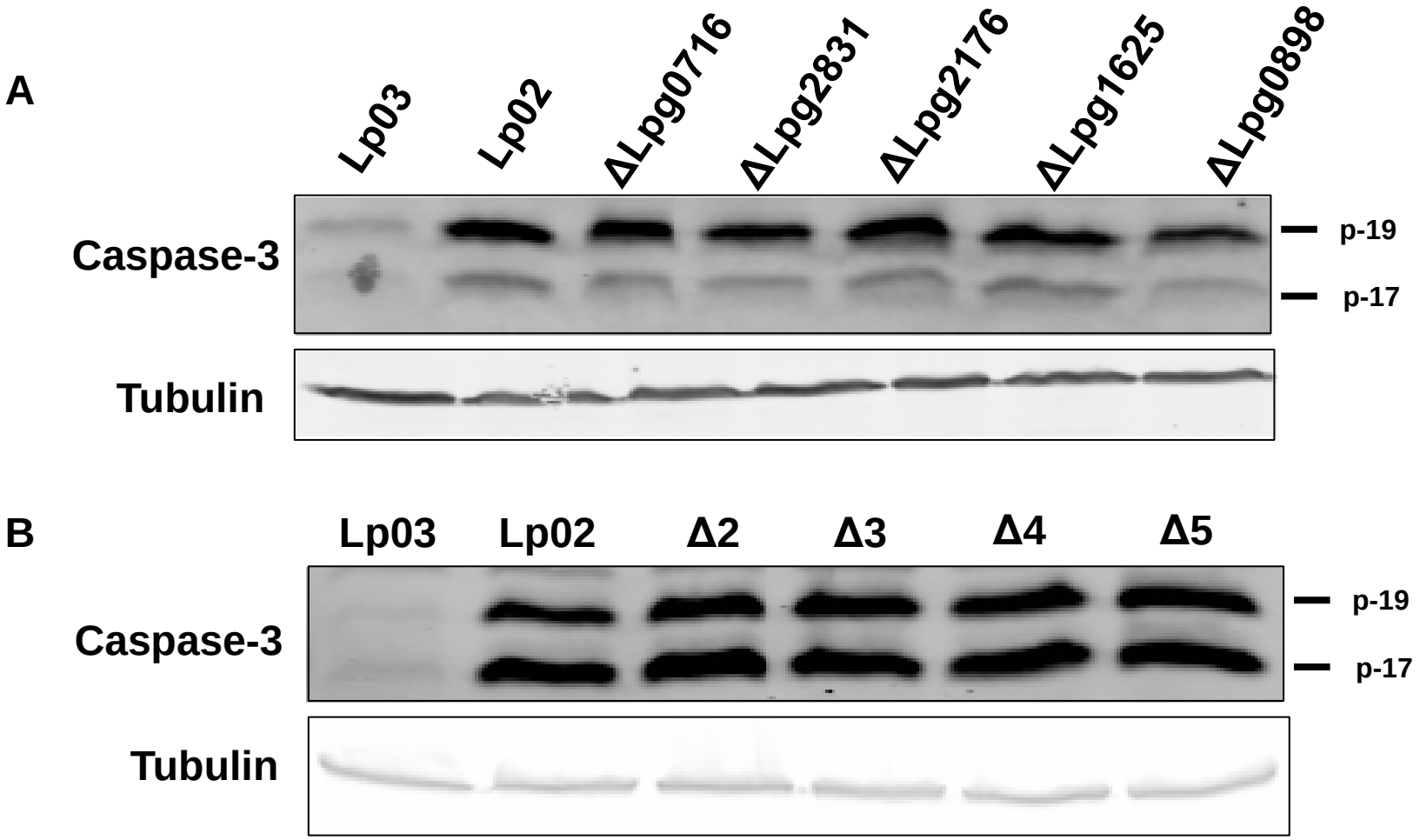

A
Lp03
Lp02
ΔLpg0716
ΔLpg2831
ΔLpg2176
ΔLpg1625
ΔLpg0898
Caspase-3
p-19
p-17
Tubulin
B
Lp03
Lp02
Δ2
Δ3
Δ4
Δ5
Caspase-3
p-19
p-17
Tubulin

Fig. S7

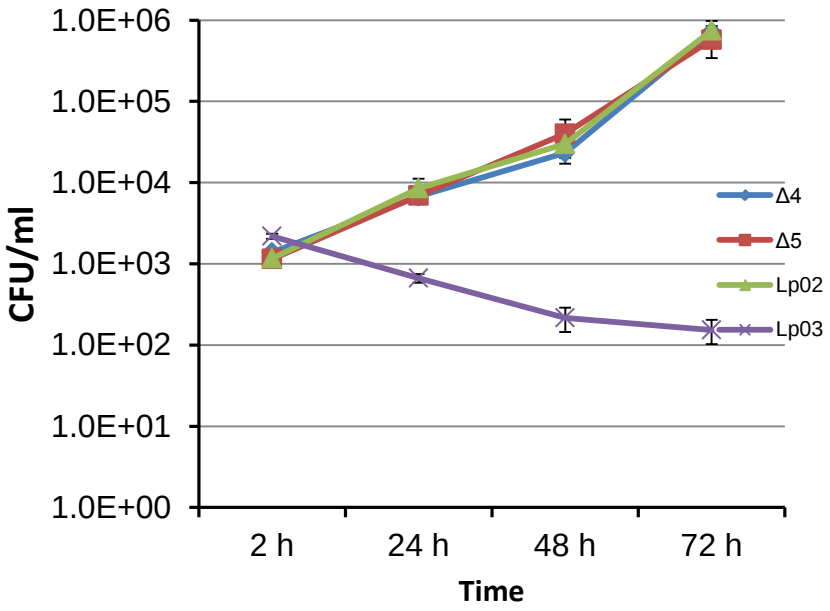

1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
1.0E+00
CFU/ml
2 h
24 h
48 h
72 h
Time
Δ4
Δ5
Lp02
Lp03

Table S1 Bacterial and yeast strains used in this study

Strains	Genotype, relevant markers	Reference
E. coli		
XL1-Blue	*recA1* endA1 *gyrA*96 *thi-1 hsd*R17 *sup*E44 *relA*1 *lac* [*F'* *proAB lacI*^{q}*ZM15* Tn*10*(Tet^{r})]	Stratagene
DH5α(λpir)	supE44 d*lacU169*(φ80*lacZ*Δ*M15*) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1 pir tet*::*Mu recA*	Our collection
L. pneumophila		
Lp02	Philadelphia-1 *rpsL hsdR thyA*	(1)
Δ 1(pJB908)	LP02Δ*lpg0898*(pJB908)	This study
Δ 2(pJB908)	LP02Δ*lpg0898*, Δ *lpg1625(*pJB908)	This study
Δ 3(pJB908)	LP02Δ*lpg0898*, Δ *lpg1625*, Δ *lpg1803*(pJB908)	This study
Δ 4(pJB908)	LP02Δ*lpg0898*, Δ *lpg1625*, Δ *lpg1803*, Δ *lpg2831* (pJB908)	(2)
Δ 5(pJB908)	LP02Δ*lpg0898*, Δ *lpg1625*, Δ *lpg1803*, Δ *lpg2831*, Δ *lpg2176* (pJB908)	This study
ΔVipD(pJB908)	Lp02Δ*vipD*(pJB908)	This study
ΔVipDfam (pJB908)	Lp02Δ*vipD*, Δ*vpdA*, Δ*vpdB*, Δ*vpdC* (pJB908)	This study
ZL14	Lp03(pJB908)	(3)
Lp03	Lp02(*dotA*$^{-}$)	(1)
Lp02(pJB908)	Lp02(pJB908)	(3)
Δ7	Chromosomal deletion strain	

Reference:

1. Berger, K. H., and Isberg, R. R. (1993) *Mol Microbiol* **7**, 7-19
2. VanRheenen, S. M., Luo, Z. Q., O'Connor, T., and Isberg, R. R. (2006) *Infect Immun* **74**, 3597-3606
3. Liu, Y., and Luo, Z. Q. (2007) *Infect Immun* **75**, 592-603

Table S2 Primers used in this study

Primer	Sequence (Restriction enzyme sites are underlined)	Note
VipD250	ctgggatccTAAATGATGCAATATTTTAC	VipD plus promoter
VipD1	ctgggatccATGAAACTTGCTGAAATTAT	VipD 5' BamHI
VipD2	ctggtcgacTTAATGGCCGCCAAATGTGG	VipD 3' SalI
VipD Δ F	GGGTTTACGGCTTTGTGGGT	VipD deletion verification primer F
VipD Δ R	TTGGATTGCGTATTTGAAC	VipD deletion verification primer R
lpg1625 up F SacI	ctggagctcAATTTTGAAGCGCCAACCGG	lpg1625 deletion upstream primer 5'
lpg1625 up R BamHI	ctgggatccCTTATCTGATTGAATGAGTT	lpg1625 deletion upstream primer 3'
lpg1625 down F BamHI	ctgggatccGCAGCCGCTGCAACAGTAGG	lpg1625 deletion downstrean primer 5'
lpg1625 down R NotI	ctggcggccgcTAAACAGTTCATTTAGACCC	lpg1625 deletion downstream primer 3'
lpg0716 up F NotI	ctggcggccgcAGTAGAAAACCAGAGTCCAA	lpg0716 deletion upstream primer 5'
lpg0716 up R BamHI	ctgggatccTGCTGTACCTGACTCGTTTT	lpg0716 deletion upstream primer 3'
lpg0716 down F BamHI	ctgggatccTCCGTATCCCCCACTATTCA	lpg0716 deletion downstream primer 5'
lpg0716 down R SalI	ctggtcgacAAGATGGATCAGTCATGGGC	lpg0716 deletion downstream primer 3'
lpg2176 up F NotI	ctggcggccgcCTCTGGCAAGGATAGTTCCT	lpg2176 deletion upstream primer 5'
lpg2176 up R BamHI	ctgggatccTAAATCTGAAACAAACCCGA	lpg2176 deletion upstream primer 3'

lpg2176 down F BamHI	ctgggatccGATGAAGGACTTAGAAATAG	lpg2176 deletion downstream primer 5'
lpg2176 down R SalI	ctggtcgacGGATTCGAACCTATGACCCT	lpg2176 deletion downstream primer 3'
lpg1803 up F SacI	ctggagctcTGTGGTGAGGGCTCGAAGCC	lpg1803 deletion upstream primer 5'
lpg1803 up R NotI	ctggcggccgcAATCAGATCACATAAAAACT	lpg1803 deletion upstream primer 3'
lpg1803 down F NotI	ctggcggccgcTTTGTTCCCAGATTGCTTAC	lpg1803 deletion downstream primer 5'
lpg1803 down R SalI	ctggtcgacTTTTTAATTGAACCAATACG	lpg1803 deletion downstream primer 3'
lpg0898 up F SalI	ctggtcgacCACAACACAGCAAAAATA	lpg0898 deletion upstream 5'
lpg0898 up R BamHI	ctgggatccAGCAGATAATATTGACAT	lpg0898 deletion upstream 3'
lpg0898 down R BamHI	ctgggatccTCATTGGCTATTCATTAA	lpg0898 deletion downstream 5'
lpg0898 down R NotI	ctggcggccgcCCAATTATCGTACCTATC	lpg0898 deletion downstream 3'
VipD S73A F	CATGTTAGCGGAGCAGCTGCCGGAGCAATGA	
VipD S73A R	GTACAATCGCCTCGTCGACGGCCTCGTTACT	
VipD D288A F	GGGTGAATACATCGCTGCTGGAGGAATTCTGGACA	
VipD D288A R	CCCACTTATGTAGCGACGACCTCCTTAAGACCTGT	

Table S3 Plasmids used in this study

Plasmid	Relevant phenotypes	Sources
pQE30	Amp	Qiagen
pJB908	Amp, *thy*$^{+}$	(1)
pEGFPC-1	For expressing C-terminal GFP fusion proteins	Clontech
pSR47S*Δ898*	Kan, for deleting *lpg0898* in *L.pneumophila* genome	This study
pSR47S*Δ1625*	Kan, for deleting *lpg1625* in *L.pneumophila* genome	This study
pSR47S*Δvipd*	Kan, for deleting *vipd* in *L.pneumophila* genome	This study
pSR47S*Δ0716*	Kan, for deleting *lpg0716* in *L.pneumophila* genome	This study
pSR47S*Δ2176*	Kan, for deleting *lpg2176* in *L.pneumophila* genome	This study
pSR47S*Δ1803*	Kan, for deleting *lpg1803* in *L.pneumophila* genome	This study
pETSUMO:*vipd*	Kan, for expression and purification of *vipd*, BamHI, SalI	This study
pETSUMO:*vipd S73A*	Kan, for expression and purification of *vipd S73A,* BamHI, SalI	This study
pEGFP898	pEGFPc1::lpg0898, BamHI, SalI	This study
pEGFP1625	pEGFPc1::lpg1625, BamHI, SalI	This study
pEGFP0716	pEGFPc1::lpg0716, BamHI, SalI	This study
pEGFP2176	pEGFPc1::lpg2176, BamHI, SalI	This study
pEGFP1803	pEGFPc1::lpg1803, BamHI, SalI	This study
pEGFPVipD	pEGFPc1::VipD, BamHI, SalI	This study
pEGFPVipDS73A	pEGFPc1::VipD$_{S73A}$, BamHI, SalI	This study

Referecne

1. Bardill, J. P., Miller, J. L., and Vogel, J. P. (2005) *Mol Microbiol* **56**, 90-103