SUPPLEMENTAL INFORMATION

Supplemental tables

TABLE S1. Strains and plasmids used in this study

Strain or plasmid	Relevant genotype or phenotype	Source or reference
Strain		
E. coli		
TOP10	F-*mcrA*, Δ(*mrr-hsdRMS-mcrBC*), ϕ80*lacZ*ΔM15, Δ*lacX*74, *recA1*, *deoR*, *araD*139, Δ(*ara-leu*)7679, *galU*, *galK*, *rpsL* (Str^R), *endA1*, *nupG*	Invitrogen
S17-1λ*pir*	*recA*, *thi*, *pro*, *hsdR*$^-$*M*$^+$, Sm^R, <RP4:2-Tc:Mu:Ku:Tn7>Tp^R	(9)
F. tularensis		
LVS	Live vaccine strain	USAMRIID[1]
Δ*iglA*	LVS, *iglA* in-frame deletion of codons 4-174	(3)
Δ*iglC*	LVS, *iglC* in-frame deletion of codons 28-205	(5)
Δ*pdpE*	LVS, *pdpE* in-frame deletion of codons 4-188	This study
Δ*iglG*	LVS, *iglG* in-frame deletion of codons 3-169	This study
Δ*iglI*	LVS, *iglI* in-frame deletion of codons 4-361	This study
Δ*vgrG*	LVS, *vgrG* in-frame deletion of codons 4-162	M. Lavander, unpublished
U112	*F. novicida*, wild-type	(1)
KKF108	U112, Δ*iglI*::*ermC*, Erm^R	(2)
S. cerevisiae		
AH109	*MATa*, *trp1-901*, *leu2-3*, *112*, *ura3-52*, *his3-200*, *gal4Δ*, *gal80Δ*, *LYS2::GAL1*$_{UAS}$-*GAL1*$_{TATA}$-*HIS3*, *GAL2*$_{UAS}$*GAL2*$_{TATA}$-*ADE2*, *URA3::MEL1*$_{UAS}$-*MEL1*$_{TATA}$-*lacZ*, *MEL1*	Clontech Laboratories
Plasmid		
pCR®4-TOPO®	TA cloning vector, Km^R, Ap^R	Invitrogen
pBluescript SK+	Cloning vector, Ap^R	Stratagene
pDM4	Suicide plasmid carrying *sacBR*, Cm^R	(7)
pJEB485	2633-bp *Xho*I/*Sac*I PCR fragment of Δ*iglA*$_{4-174}$ with flanking regions on pDM4, Cm^R	(3)
pJEB750	2469-bp *Xho*I/*Sac*I PCR fragment of Δ*pdpE*$_{4-188}$ with flanking regions on pDM4, Cm^R	This study
pJEB751	2517-bp *Xho*I/*Sac*I PCR fragment of Δ*iglI*$_{4-361}$ with flanking	This study

	regions on pDM4, Cm^R	
pJEB753	2425-bp *Xho*I/*Sac*I PCR fragment of Δ*iglG*$_{3\text{-}169}$ with flanking regions on pDM4, Cm^R	This study
pKK289Km	Expression plasmid carrying a *gfp* gene under the control of the LVS GroESL promoter, Km^R	(4)
pMOL103	pKK289Km encoding IglG-6xHis fusion protein, Km^R	This study
pMOL42	pKK289Km carrying pUC19 MCS from *Hind*III to *Eco*RI, with an upstream *Nde*I site, Km^R	This study
pMOL52	pKK289Km derivative used to construct C-terminal fusion proteins to eukaryotic GSK, Km^R	This study
pMOL59	pMOL52 encoding IglI-GSK fusion protein, Km^R	This study
pMOL61	pMOL52 encoding PdpE-GSK fusion protein, Km^R	This study
pKEK1012	pKEK894 encoding VgrG-CyaA fusion protein, Tet^R	(2)
pKEK1051	pKEK894 encoding IglI-CyaA fusion protein, Tet^R	(2)
pJEB835	pKEK894 encoding IglG-CyaA fusion protein, Tet^R	This study
pJEB851	pKEK894 encoding IglI (LVS)-CyaA fusion protein, Tet^R	This study

[1] US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD, USA.

1 TABLE S2. Oligonucleotides used in this study

Purpose	Oligonucleotide pair(s)
LVS null mutants	
PdpE Δ4-188 (pJEB750)	PdpE_a: 5´-*CTC GAG* GTA AGA TCG GAG TTG ATT CTA A-3´ (*Xho*I) and PdpE_b: 5´-*GGA TCC* ACT ATA ATA TAA CTC TAG TTA AGA A-3´ (*Bam*HI)
	PdpE_c: 5´- *GGA TCC* TTT ACT CAT ATA TTT GTA TCC TTA A -3´ (*Bam*HI), PdpE_d: 5´-*GAG CTC* ATA CGC GAT ATT GCT ACG GA-3´ (*Sac*I)
IglG Δ3-169 (pJEB753)	IglG_a: 5´-*CTC GAG* GAA TAC ATA TTC TTA GAG ATA ATC-3´ (*Xho*I) and IglG_b: 5´-*GGA TCC* GTA AAA ACA TCT TAG AAG GTC AT-3´ (*Bam*HI)
	IglG_c: 5´-*GGA TCC* TAA CAT TTA AAT TTT CCA ATA AGC T-3´ (*Bam*HI) and IglG_d: 5´-*GAG CTC* AAA TCA TAT TTC GAT ACG CTC A-3´ (*Sac*I)
IglI Δ4-361 (pJEB751)	IglI_a: 5´-*CTC GAG* GGC ATA AAT GAT GTT AAC TTA TG-3´ (*Xho*I) and IglI_b: 5´-*GAT ATC* AAT GAC CCC GTA GAA AAA ATT TC-3´ (*Eco*RV)
	IglI_c: 5´-*GAT ATC* CTG ACT CAT ATA AAT CTC CTC-3´ (*Eco*RV) and IglI_d: 5´-*ACT AGT* AGT ATT ATC TGT TTA TGA GAA TAA T-3´ (*Spe*I)
Complementation	
IglG-6xHis (pMOL103)	pigD_for: 5´-*CAT ATG* TTA AAT ATT ATA AAT GAC TCC-3´ (*Nde*I) and pigD_6xH_rev_C: 5´-*GAA TTC* CTA ATG ATG ATG ATG ATG ATG AGA TGT TTT TAC ATT TAT TTG TC-3´ (*Eco*RI)
IglI-GSK (pMOL59)	pigG_for: 5´-*CAT ATG* AGT CAG ATA ATA TCT ACA C-3´ (*Nde*I) and pigG_gsk_rev: 5´- *GGT ACC* TAT GTC AAA AAG ATC TTC AAA ATA -3´ (*Kpn*I)
PdpE-GSK (pMOL61)	pigI_for: 5´-*CAT ATG* AGT AAA AAA ATA TTT AAA TTA TTA-3´ (*Nde*I) and pigI_gsk_rev: 5´-*GGT ACC* TAT TAT AGT AAT TTT CTT TTC ATA AT-3´ (*Kpn*I)
IglG-CyaA (pJEB835)	IglG_Cya_F: 5´-*CCA TGG* AAA TGT TAA ATA TTA TAA ATG ACT CCT T-3´ (*Nco*I) and IglG_Cya_R: 5´-*CAT ATG* AGA TGT TTT TAC ATT TAT TTG TCC ACT A-3´ (*Nde*I)
IglI-CyaA (pJEB851)	IglI_Cya_F: 5´-*CCA TGG* AAA TGA GTC AGA TAA TAT CTA CAC TA-3´ (*Nco*I) and IglI_Cya_R: 5´-*CAT ATG* TAT GTC AAA AAG ATC TTC AAA ATA GT -3´ (*Nde*I)

1 The nucleotide sequences in italics represent the incorporated *Nde*I, *Eco*RI, *Bam*HI, *Sac*I, *Xho*I, *Kpn*I, *Eco*RV, *Spe*I, and *Nco*I restriction sites used for cloning of

2 the PCR amplified DNA fragments.

TABLE S3. Quantitative real time PCR analysis of Δ*pdpE*, Δ*iglG* and Δ*iglI* mutants

Gene	Strain		
	Δ*pdpE*	Δ*iglG*	Δ*iglI*
pdpA	1.80 ± 0.83	0.89 ± 0.45	1.43 ± 0.73
icmF	1.76 ± 0.59	1.62 ± 0.46	1.91 ± 0.75
iglE	1.83 ± 0.75	1.23 ± 0.68	2.07 ± 0.56
vgrG	1.76 ± 0.77	1.25 ± 0.97	2.03 ± 1.05
iglF	1.41 ± 0.53	2.04 ± 0.85	1.67 ± 0.48
iglG	1.27 ± 0.07*	NA	1.06 ± 0.15
iglH	1.39 ± 0.34	0.80 ± 0.43	1.36 ± 0.47
dotU	1.69 ± 0.75	1.26 ± 0.66	1.27 ± 0.13
iglI	1.88 ± 0.63	1.56 ± 0.41	NA
iglJ	1.57 ± 0.60	1.71 ± 0.60	2.14 ± 0.72
pdpC	1.47 ± 0.70	1.32 ± 0.60	1.17 ± 0.53
pdpE	NA	1.12 ± 0.43	1.83 ± 0.34
iglD	0.81 ± 0.25	0.90 ± 0.48	0.87 ± 0.42
iglC	1.21 ± 0.22	0.74 ± 0.18	1.18 ± 0.18
iglB	1.45 ± 1.00	0.95 ± 0.48	1.47 ± 1.09
iglA	1.74 ± 0.78	1.25 ± 0.10	1.80 ± 1.04
pdpD	1.24 ± 1.31	1.59 ± 1.00	2.15 ± 1.43

The N-fold differential expression of each target gene in *ΔiglG*, *ΔiglI* or *ΔpdpE* mutants compared to the relative expression of the same gene in LVS was defined as $2^{-\Delta\Delta CT}$ (6). As before (8), the range of mRNA expression was defined by the N-fold change as follows: overexpressed (N-fold change ≥ 2.0, gray box) or underexpressed (N-fold change ≤ 0.5). Data are mean ± standard deviation of 3 independent experiments. A 2-sided paired Student´s *t*-test was used to estimate if gene expression was significantly different in mutants compared to LVS. * $P < 0.05$. NA = not applicable.

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