Supplementary Data

Table S1: *Listeria monocytogenes* strains used in this study.

Strain	Strain#	Description	Source
WT	-	Wild type *Listeria monocytogenes* 10403S strain	(1)
Δ*secA2*	DP-L4342	In frame deletion of *lmo0583*	(2)
Δ*pgdA*Δ*oatA*	DP-L5220	In frame deletion of *lmo0415* and *lmo1291*	(3)
Δ*iap*	DP-L4611	In frame deletion of *lmo0582*	(4)
Δ*lmo2769*	DP-L6209	In frame deletion of *lmo2769*	This study
Δ*lmo2769*Δ*secA2*	DP-L6210	In frame deletion of *lmo2769* and Δ*secA2*	This study
Δ*lmo2769*Δ*secA2*+pPL2:2767-2769	DP-L6211	Δ*lmo2769*Δ*secA2strain* complemented with pPL2 *lmo2767-2769* construct	This study
R57+pPL2:2767-2769	DP-L6212	R57 strain complemented with pPL2 *lmo2767-2769* construct	This study
lmo2768::Tn	DP-L6155	*Himar1* Transposon insert in *lmo2768*	(5)
ΔsecA2:*lmo2768*::Tn	DP-L6213	Δ*secA2* strain with *Himar1* Transposon insert in *lmo2768*	This study
Δ*lmo1721*	DP-L6214	In frame deletion of *lmo1721*	This study
Δ*lmo1721*Δ*secA2*	DP-L6215	In frame deletion of *lmo1721* and *secA2*	This study
Δ*lmo2769*Δ*lmo*1721	DP-L6216	In frame deletion of *lmo1721* and *lmo2769*	This study
Δ*lmo2769*Δ*lmo1721*Δ*secA2*	DP-L6217	In frame deletion of *lmo1721, lmo2769* and *secA2*	This study
Δ*secA2:lmo2637*::Tn	DP-L6218	*Himar1* Transposon insert in *lmo2637* in Δ*secA2* strain **with** WT *secY*	This study
Δ*secA2prlA1*	DP-L6219	*Himar1* Transposon insert in *lmo2637* in Δ*secA2* strain **with** *secY* (G408R)	This study
*lmo*2637::Tn	DP-L6220	*Himar1* Transposon insert in *lmo2637* in 10403S (WT) strain **with** WT *secY*	Lab collection
prlA1	DP-L6221	*Himar1* Transposon insert in *lmo2637* in WT strain **with** *secY* (G408R)	This study
Δ*lmo2769*Δ*secA2:lmo2637*::Tn	DP-L6222	*Himar1* Transposon insert in *lmo2637* in Δ*lmo*2769Δ*secA2* strain **with** WT *secY*	This study
Δ*lmo2769*Δ*secA2prlA1*	DP-L6223	*Himar1* Transposon insert in *lmo2637* in Δ*lmo*2769Δ*secA2* strain **with** *secY* (G408R)	This study
Δ*lmo1721*Δ*secA*2*prlA1*	DP-L6224	*Himar1* Transposon insert in *lmo2637* in Δ*lmo1721*Δ*secA2* strain **with** *secY* (G408R)	This study
Δ*lmo2769*Δ*lmo1721*Δ*secA2*::Tn	DP-L6225	*Himar1* Transposon insert in *lmo2637* in Δ*lmo2769*Δ*lmo1721*Δ*secA2* strain **with** WT *secY*	This study
Δ*lmo2769*Δ*lmo1721*Δ*secA2prlA1*	DP-L6226	*Himar1* Transposon insert in *lmo2637* in Δ*lmo2769*Δ*lmo1721*Δ*secA2* strain **with** *secY* (G408R)	This study
R57*prlA1*	DP-L6227	*Himar1* Transposon insert in *lmo2637* in strain R57 **with** the *secY* (G408R)	This study
R57:*lmo2637*::Tn	DP-L6228	*Himar1* Transposon insert in *lmo2637* in strain R57 **with** WT *secY*	This study

Table S2: Deletion and complement primer sequences.

Primer	5'-3' Sequence	Description
JD17	ATTAGTCGACCTCGGAGTTTGGTGTCTTCTGG	*lmo2769* deletion primer A
JD18	ATTACTGCAGAAACGATGCGGACTCAAACG	*lmo2769* deletion primer D
JD19	CTCCCGTCTGTTTTAAATCTCGTATTTAGTTAAGTTCCGAATTTTCAT	*lmo2769* deletion primer B
JD20	ATGAAAATTCGGAACTTAACTAAA TACGAGATTTAAAACAGACGGGAG	*lmo2769* deletion primer C
JD30	ATTAGTCGAC ACAGATGTAGCGGCTCGTGG	*lmo1721* deletion primer A
JD31	GATTCCTTTTTCTTAATTTTCTTCGACTTCTTCTTTTCTACTAGACAT	*lmo1721* deletion primer B
JD32	ATGTCTAGTAGAAAAGAAGAAGTCGAAGAAAATTAAGAAAAAGGAATC	*lmo1721* deletion primer C
JD33	ATTACTGCAGCGCCGTCCATTGTTCCATAG	*lmo1721* deletion primer D
JD46	ATTAGTCGACCTGGATGTGGCGTAAGGG	*lmo2769-lmo2767* F' primer
JD47	ATTAGGATCCCATAACTTTGTCCCGATTGTCC	*lmo2769-lmo2767* R' primer

Table S3: **Colony and microscopic morphology of *ΔsecA2* revertants.**

Strain	Colony	Chaining[#]	Lysozyme[$]	Strain	Colony	Chaining[#]	Lysozyme[$]
R1	smooth	+	1.79 (±0.10)	**R33**	rough	+++	1.71 (±0.00)
R2	rough	++++	1.64 (±0.10)	**R34**	smooth	++[€]	2.67 (±0.20)
R3.1	smooth	+	1.71 (±0.00)	**R35**	smooth	++	2.67 (±0.60)
R3.2	rough	++++	1.71 (±0.20)	**R36**	smooth	+	1.64 (±0.10)
R4	smooth	+	1.64 (±0.10)	**R37**	smooth	++	1.71 (±0.00)
R5	smooth	++	1.71 (±0.00)	**R38**	smooth	++	1.57 (±0.00)
R6	rough	+++	1.57 (±0.00)	**R39**	rough	++++	2.00 (±0.20)
R7	smooth	+	2.36 (±0.10)	**R40**	rough	+++[€]	1.79 (±0.10)
R8	rough	+++	2.50 (±0.10)	**R41**	smooth	+	1.79 (±0.10)
R9	rough	+++	2.17 (±0.30)	**R42**	smooth	+	2.14 (±0.20)
R10.1	smooth	++	1.71 (±0.00)	**R43**	rough	+++	1.57 (±0.20)
R10.2	rough	+++	1.86 (±0.00)	**R44**	smooth	+	2.21 (±0.30)
R11	smooth	++[€]	1.86 (±0.00)	**R45**	smooth	+	2.29 (±0.20)
R12	smooth	++	2.29 (±0.00)	**R46**	smooth	+	2.50 (±0.10)
R13	rough	+++	1.64 (±0.10)	**R47.1**	smooth	+	2.57 (±0.00)
R14	rough	+++	1.93 (±0.10)	**R47.2**	rough	+++	2.64 (±0.10)
R15	rough	+++	1.86 (±0.00)	**R48**	smooth	+	1.71 (±0.00)
R16	smooth	+	1.71 (±0.00)	**R49**	smooth	++	1.71 (±0.20)
R17	smooth	++[€]	1.71 (±0.00)	**R50**	smooth	++	2.21 (±0.10)
R18.1	smooth	+[€]	1.71 (±0.00)	**R51**	rough	+	1.71 (±0.00)
R18.2	rough	++++	1.64 (±0.10)	**R52**	smooth	+	1.64 (±0.10)
R19	rough	++++	1.79 (±0.10)	**R53**	smooth	+	2.29 (±0.20)
R20	rough	+++	1.79 (±0.10)	**R54**	smooth	++	2.57 (±0.20)
R21	smooth	++	1.93 (±0.10)	**R55**	rough	+++	1.86 (±0.40)
R22	rough	+++	1.79 (±0.10)	**R56**	smooth	++	2.14 (±0.00)
R23	smooth	+	2.10 (±0.40)	**R57**	smooth	+	2.87 (±0.40)
R24	smooth	+[€]	2.14 (±0.20)	**R58**	smooth	+	1.86 (±0.20)
R25	rough	++++	1.64 (±0.10)	**R59**	smooth	+	1.79 (±0.10)
R26	smooth	+[€]	2.93 (±0.30)	**R60.1**	smooth	++	2.49 (±0.20)
R27	rough	+++	1.64 (±0.10)	**R60.2**	rough	+++	1.71 (±0.00)
R28	smooth	++	1.71 (±0.00)	**R61**	smooth	+	2.29 (±0.00)
R29	smooth	+	1.71 (±0.20)	**R62**	smooth	++	2.50 (±0.10)
R30	smooth	+	3.36 (±0.10)	**R63.1**	smooth	++	2.21 (±0.10)
R31	rough	+++	1.79 (±0.10)	**R63.2**	rough	+++	2.21 (±0.10)
R32	smooth	++[€]	1.64 (±0.10)	**R64**	smooth	+[€]	3.00 (±0.20)

[#] Microscopy was conducted on stationary phase cells grown in BHI at 37°C. Chaining phenotype was scored from + to ++++, where WT = + and *ΔsecA2* =++++ according to the following algorithm:

+ = single cells and chains of up to 3 cells/field x40 magnification
++ = single cells and chains of up to 4 cells/field x40 magnification
+++ = chains of up to 6 cells/field x40 magnification
++++ = chains of >6 cells/field x40 magnification

[€] Pleomorphic cells

[$] Disk diffusion susceptibility to 1mg of lysozyme expressed as ratio of WT, where a ratio >1 indicates increased susceptibility. Values in parenthesis represent standard deviations.

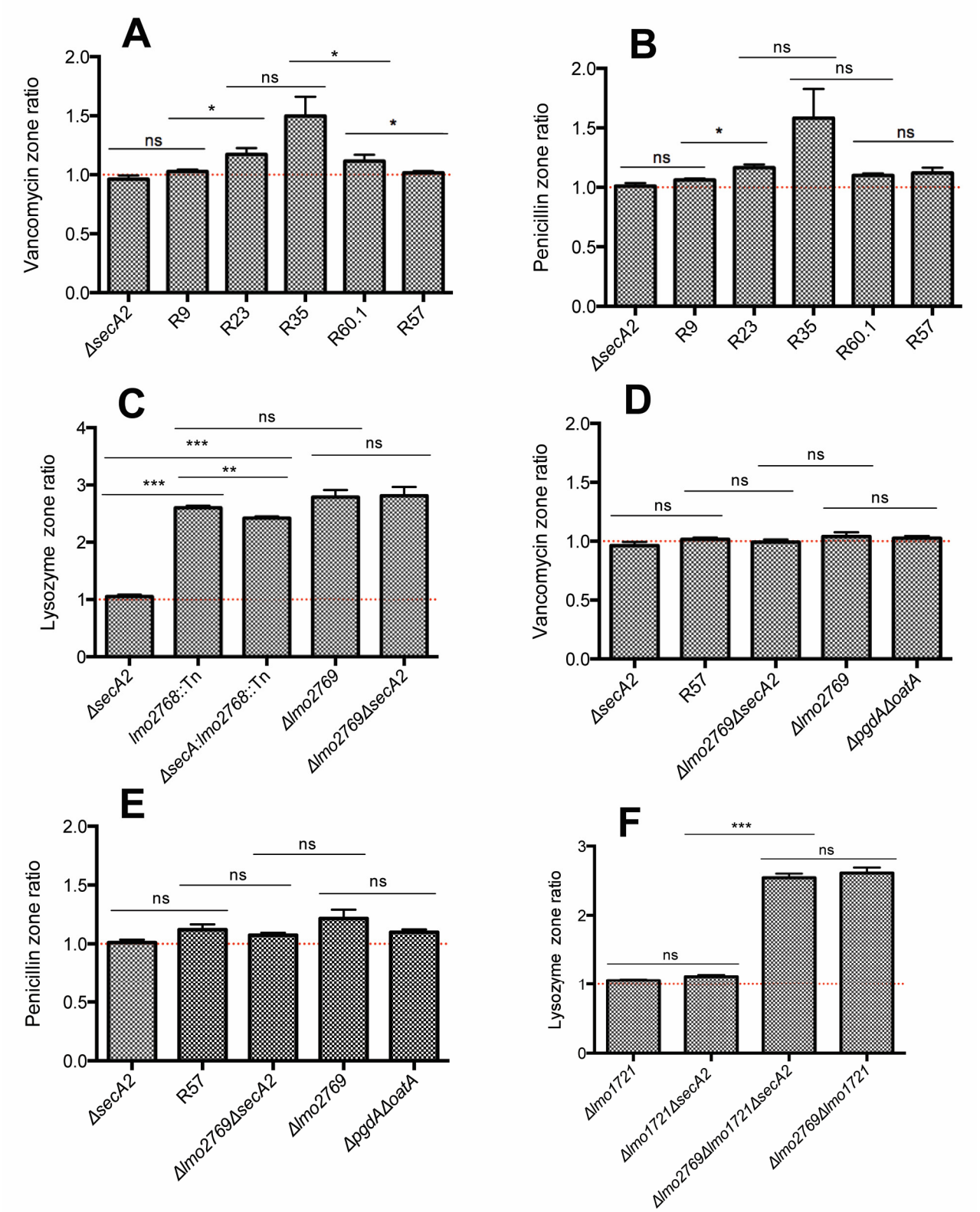

Figure S1: A and D) Disk diffusion susceptibility to 10 µg of vancomycin, B and E) 120 µg of penicillin and C and F) 1mg of lysozyme expressed as ratio of WT, where a ratio >1 indicates increased susceptibility. Error bars represent standard deviations of the mean zone ratio of WT. Student's t- test was used to analyze statistical significance where ***, $P < 0.0001$ and ns- $P \geq 0.05$.

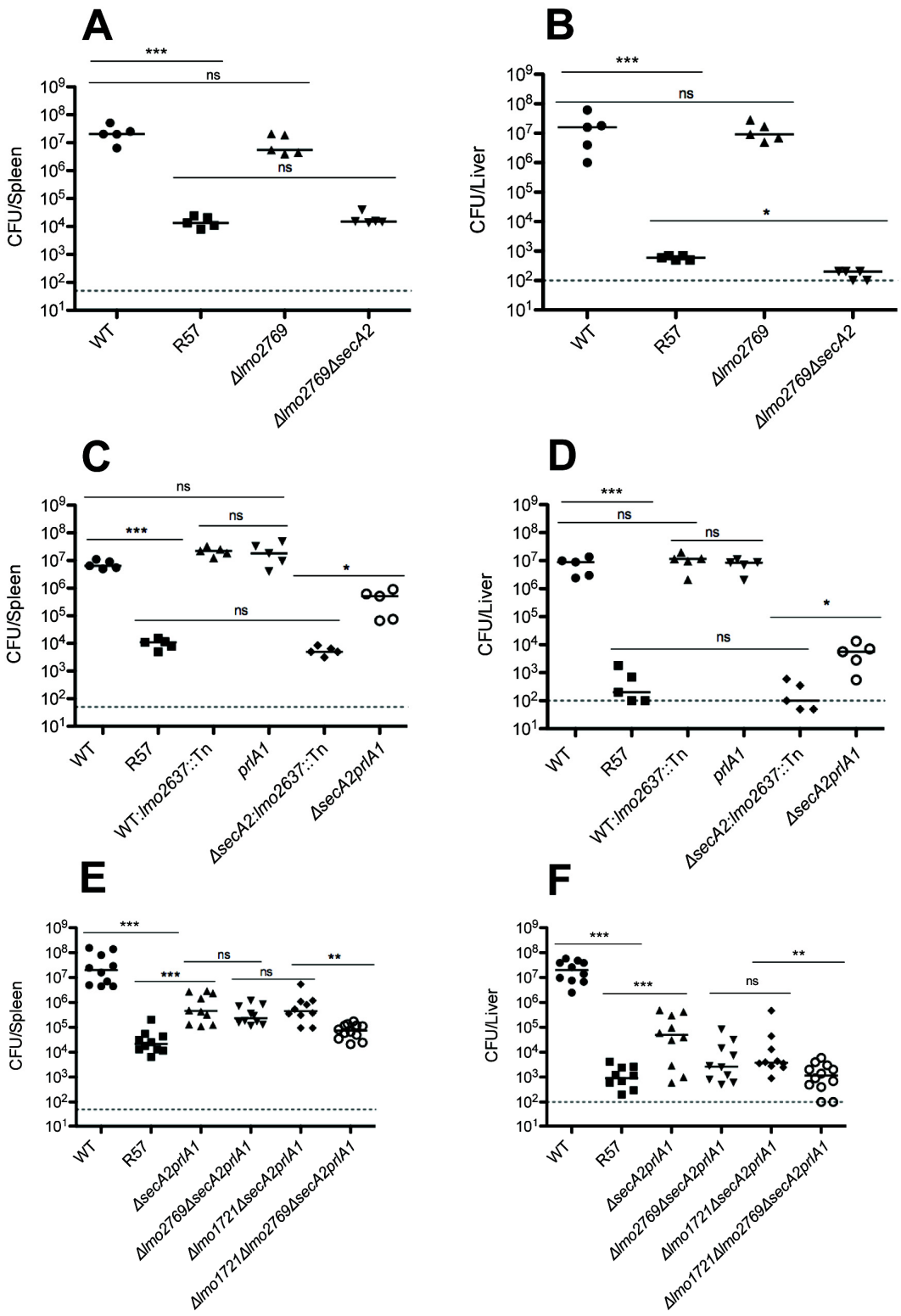

Figure S2: *In vivo* virulence of assorted mutants. Bacterial burdens in spleens and livers 48hr post infection with $1x10^5$ CFU in CD1 mice. The dashed-line represents the limit of detection. Statistical significance was evaluated using Mann Whitney test. *** $P < 0.0001$, ns - $P \geq 0.05$.

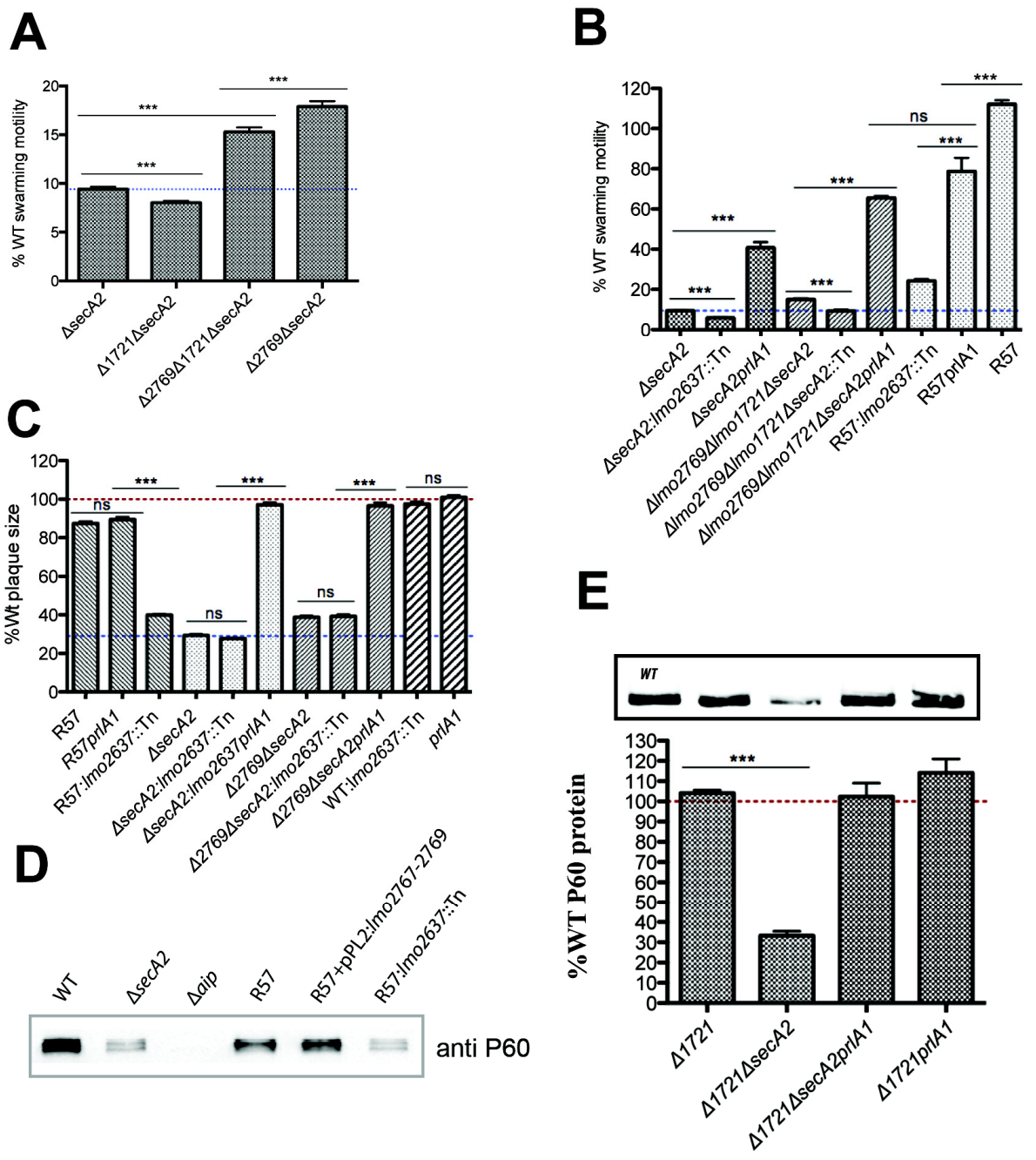

Figure S3: Swarming motility of *lmo*1721 A) and *lmo*2637::Tn B) mutants was assessed on semi-solid LB agar following incubation at 30°C after 48hr. Motility is expressed as a percentage of swarming area of the WT strain. Error bars represent standard deviations of the mean zone ratio between three independent experiments. C) Plaque area of each mutant is shown as a percentage when compared to the WT strain. Error bars represent standard deviations from the mean between three independent experiments. Student's t- test was used to analyze statistical significance where ***, P< 0.0001; *, P< 0.05 and ns- P ≥ 0.05. D) An image of a western blot showing P60 in supernatants from mid-log (5h) cultured cells in LB broth. E) Secreted levels of P60 in supernatants from mid-log (5h) cultured cells in LB broth expressed as a percentage of protein secreted by the WT strain.

References

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