

Figure S1

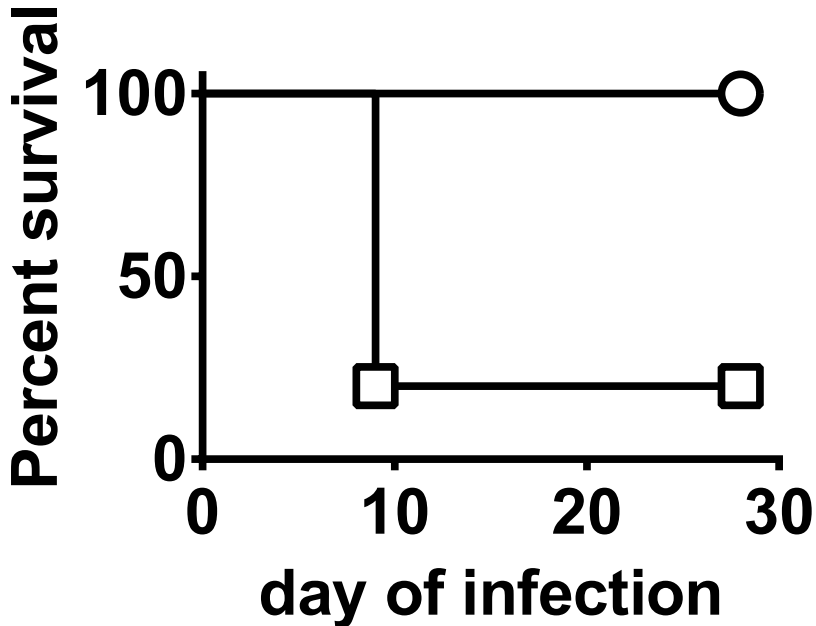


Figure S2

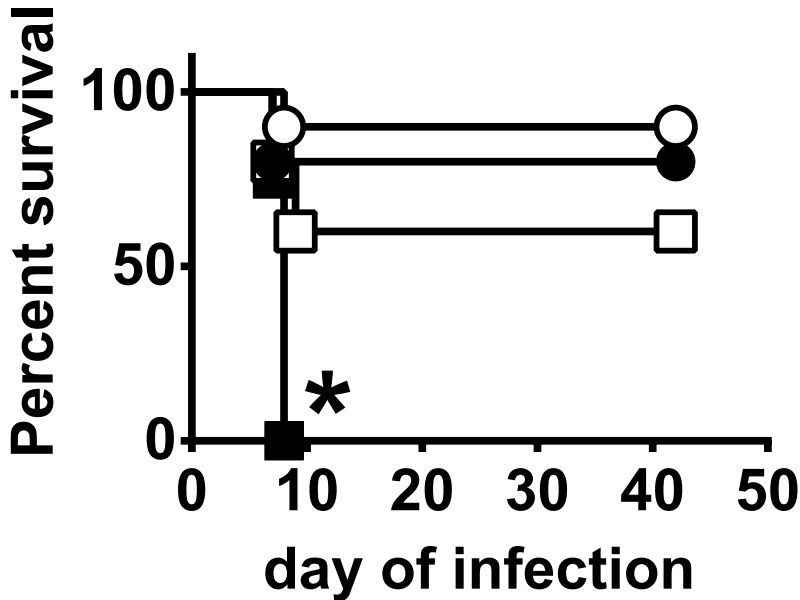


Figure S3

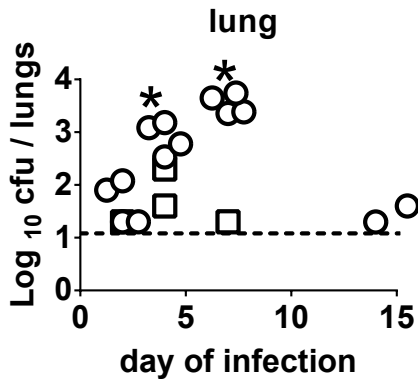
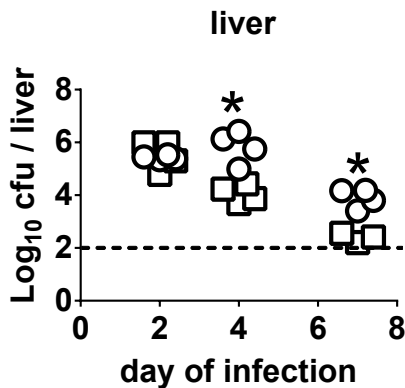
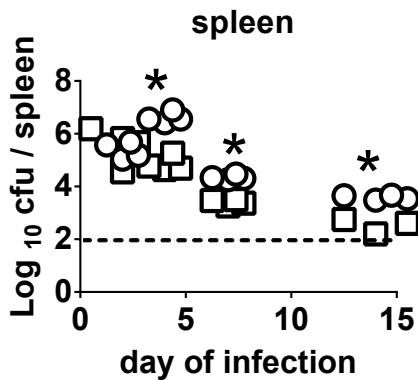
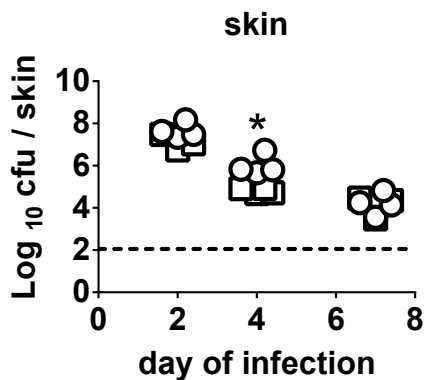


Figure S4

Francisella Skin Reaction Score Chart

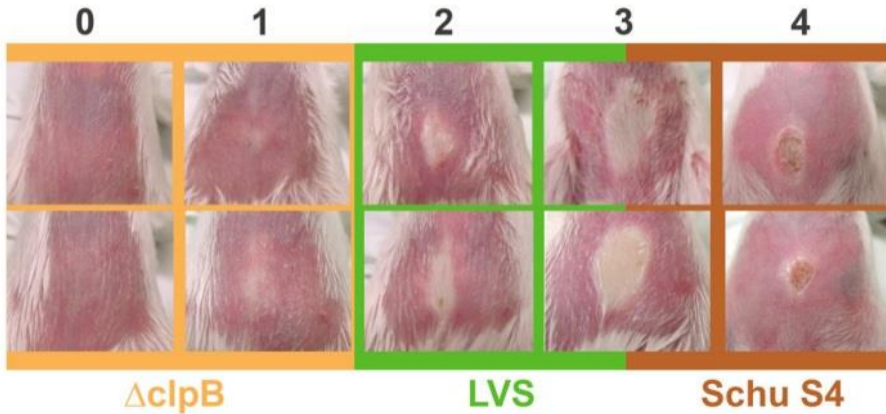


Figure S5

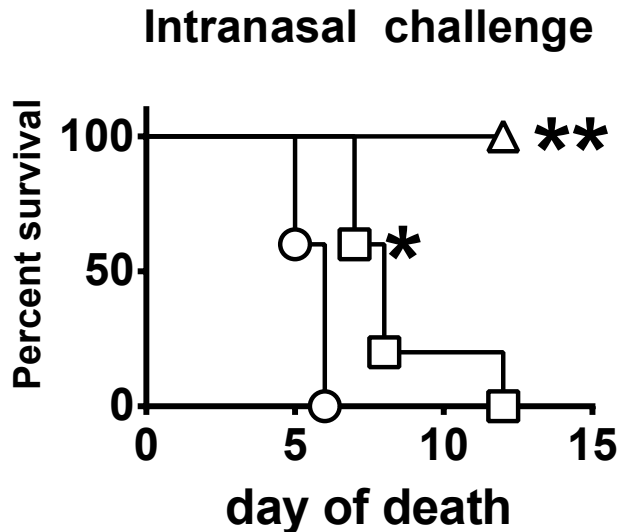
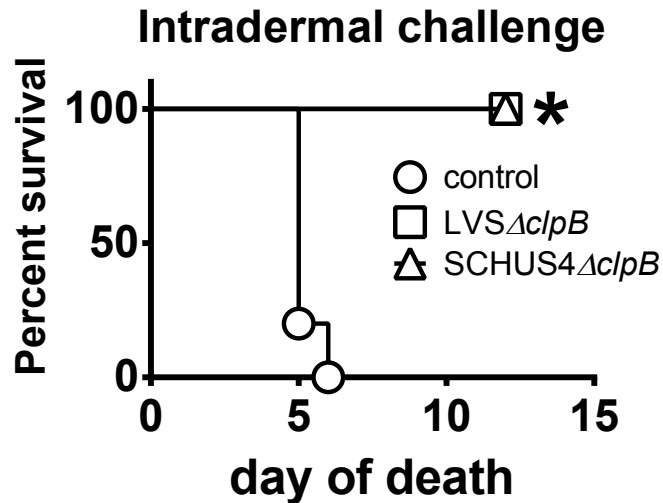


Table S1. Growth of attenuated strains of *F. tularensis* in SCID mice

Bacteria	Log ₁₀ CFU /organ ± SD				
	Lung	Liver	Skin	spleen	Blood
SCHUS4Δ <i>clpB</i>	5.63 ±0.54	6.77 ±0.21*	6.15 ±0.37	6.47 ±0.13	4.13 ±0.37
FSC200Δ <i>clpB</i>	6.41± 0.92*	7.27 ±0.62*	7.37± 1.16 *	7.35± 0.36**	5.52± 0.97
LVS	5.14± 0.57	5.65± 0.51	5.35± 0.34	6.03± 0.41	4.42± 0.74

SCID mice (n=5 /group) were challenged ID with 10³ CFU of one or other bacterial strain, and killed on day 8 of infection for bacteriology. *, significantly greater burden than LVS, **, significantly greater burden than SCHUS4Δ*clpB* or LVS

Table S2. Attenuation and immunogenicity profiles of selected SCHU S4 deletion mutants administered ID or IN to BALB/c mice.

Strain	ID LD ₅₀	Protection against ID challenge ¹		Protection against aerosol challenge ¹	
		Vaccine Dose.	Protection	Vaccine Dose.	Protection
<i>ΔwbtC</i>	~10 ⁷	10 ³	++++	10 ⁷	-
<i>ΔpmrA</i>	~10 ¹	10 ¹	++++	ND	ND
<i>ΔrelA</i>	<10 ¹	ND	ND	ND	ND
<i>ΔfupA</i>	~10 ⁴	10 ³	++++	10 ³	+
<i>ΔclpB</i>	>10 ⁷	10 ³ , 10 ⁵	++++	10 ³ , 10 ⁷	+++
<i>Δggt</i>	<10 ⁷	10 ³	++++	10 ⁵	+++
<i>ΔcapB</i>	<10 ¹	ND	ND	ND	ND
<i>ΔclpBΔpmrA</i>	>10 ⁷	ND	ND	10 ⁷	++
<i>ΔclpBΔrelA</i>	>10 ⁷	ND	ND	10 ⁷	-
<i>ΔclpBΔcapB</i>	>10 ⁷	10 ⁷	++++	10 ⁷	++
<i>ΔclpBΔwbtC</i>	>10 ⁷	10 ⁷	++++	10 ⁷	-
<i>ΔclpBΔggt</i>	>10 ⁷	10 ³	+++	10 ⁷	+
<i>ΔclpBΔfupA</i>	>10 ⁷	10 ⁷	++++	10 ⁷	+
LVS	>10 ⁷	10 ³ , 10 ⁶	++++	10 ³ , 10 ⁵ , 10 ⁶ , 10 ⁷	+

Mice were challenged ≥ 4 weeks post-vaccination.

¹, the ID challenge dose was 1,000 SCHUS4 and the aerosol dose~ 100 CFU. All naïve mice died within 6 days of ID or IN challenge.

- ++++ all vaccinated mice survived challenge for >21 days
- +++ at least 50% of vaccinated mice survived challenge for >21 days
- ++ ≤ 20% vaccinated mice survived challenge for >21 days
- + mice survived significantly longer than naïve mice, but all died in <21 days
- Mice did not survive significantly longer than naïve mice
- ND Not determined