Supplemental Figure Legends

Supplemental Fig S1. *In vitro* replication rates of *B. mallei* WT and mutant strains. Panel A: Plate-grown bacteria were suspended in PBS and used to infect *n*=2 wells of duplicate tissue culture plates seeded with murine J774 macrophages (multiplicity of infection=10:1). The infected cells were incubated for 1 hr at 37^{o}C to allow phagocytosis of bacteria, washed, and treated with antibiotic for 2 hours at 37^{o}C to kill extracellular organisms. Cells from one tissue culture plate were lysed, diluted, and plated onto agar medium to determine the number of bacteria phagocytized. The other tissue culture plate was incubated for an additional 7 hours, time after which cells were washed, lysed, diluted, and spread onto agar plates to calculate the number of intracellular organisms. The results are expressed as the mean (± standard error) intracellular replication index, which was calculated by dividing the number of intracellular bacteria at the end point of the assay (second tissue culture plate) by the number of bacteria phagocytized (first tissue culture plate). These assays were performed on 6 separate occasions. The asterisk indicates that the reduction in the intracellular replication index of the *batA* KO mutant is statistically significant using a paired *t* test (*P*=0.007). Panel B: Plate-grown bacteria were suspended in broth to an optical density at wavelength 600 nm (OD_{600}) of 0.1. Following this, bacteria were incubated at 37°C with shaking (200-rpm) and the optical density of cultures was measured from duplicate samples at the indicated time intervals. Strains were tested on 2 separate occasions; the results of one experiment are shown. Error bars correspond to standard error of the mean.

Supplemental Fig S2. Median lethal dose comparison of *B. mallei* WT and mutant strains. BALB/c mice were inoculated intratracheally using a Microsprayer device to aerosolize the indicated number of bacterial CFU into the lungs (*n*=5 mice/dose). Animals were then

monitored daily for clinical signs of illness and morbidity. Panel A: Kaplan-Meier survival curves. Panel B: Calculated LD_{50} values (per Reed and Muench method). Panels C and D: Tissues were collected from mice that survived challenge (day 15), homogenized, diluted, and spread on agar plates to determine bacterial loads. Symbols show data for individual animals; horizontal bars represent the mean total CFU for each group.

Supplemental Fig S3. Kinetics of bacterial accumulation in target tissues of mice vaccinated the *batA* KO mutant strain. BALB/c mice were vaccinated intratracheally with 10^4 CFU of the *batA* KO strain using a Microsprayer device. Thirty days later, the animals were back-challenged with ~10LD_{50} of WT *B. mallei* ATCC 23344. Age and weight-matched naïve BALB/c mice were used as controls. At the indicated time points post-inoculation, tissues were collected from *n*=3 mice, homogenized, diluted, and spread on agar plates to determine bacterial loads. Symbols show data for individual animals; horizontal bars represent the mean total CFU for each group. These experiments were performed on 2 separate occasions and the graphs show cumulative results.

Supplemental Fig S4. Kinetics of bacterial accumulation in target tissues of mice that were administered immune serum (from mice vaccinated with the *batA* KO strain). BALB/c mice were administered 1-mL of immune or naïve serum IP. Forty-eight hours later, the animals were challenged intratracheally with ~10LD_{50} of WT *B. mallei* ATCC 23344 using a Microsprayer device. At the indicated time points post-inoculation, tissues were collected from *n*=3 mice, homogenized, diluted, and spread on agar plates to determine bacterial loads. Symbols show data for individual animals; horizontal bars represent the mean total CFU for each group. These experiments were performed on 2 separate occasions and the graphs show cumulative results.

Supplemental Fig S5. Reactivity of immune serum fractions with whole bacteria, capsular polysaccharides (CPS) and oligosaccharide chains of LPS (OPS). Serum and antibody samples were serially diluted and placed in duplicate wells of plates coated with paraformaldehyde fixed *B. mallei* ATCC 23344 bacteria (panel A), CPS (panel B), or OPS (panel C). Alkaline phosphatase-conjugated, goat α-mouse (Ig H+L) antibodies were used for detecting total binding antibodies. The y-axis shows absorbance at a wavelength of 405 nm. The x-axis represents serial two-fold dilutions of samples. The results are expressed the mean absorbance (± standard error). Reciprocal end-point titers are indicated by arrows and correspond to the highest fold dilutions of serum samples giving an absorbance reading at a wavelength of 405 nm greater than the mean absorbance value plus 3 standard deviations of wells probed with naïve serum. Serum and antibody samples are described in details in Fig 8A.

Supplemental Fig S6. Opsonophagocytic killing assays using WT *B. mallei* and purified IgG antibodies. Panels A and B: Freshly-grown *B. mallei* ATCC 23344 bacteria were incubated with purified IgG antibodies for 30 min at 37^{o}C, and the opsonized organisms were used to infect *n*=2 wells of duplicate tissue culture plates seeded with murine J774 macrophages. The infected cells were incubated for 1 hr at 37^{o}C to allow phagocytosis of bacteria, washed, and treated with antibiotic for 2 hours at 37^{o}C to kill extracellular organisms. Cells from one tissue culture plate were lysed, diluted, and plated onto agar medium to determine the number of bacteria phagocytized. The results are shown in Panel A and are expressed as the mean CFU (± standard error). The other tissue culture plate was incubated for an additional 7 hours, time after which cells were washed, lysed, diluted, and spread onto agar plates to calculate the number of intracellular organisms. The results are shown in Panel B and are expressed as the mean (± standard error) intracellular replication index, which was calculated by dividing the number of

intracellular bacteria at the end point of the assay (second tissue culture plate) by the number of bacteria phagocytized (first tissue culture plate); an index below 1 (blue dotted line) indicates intracellular killing of bacteria. These assays were performed on 5 separate occasions. Bacteria incubated with PBS and IgG antibodies purified from naïve serum (prior to infecting macrophages) were used as controls. *P* values indicate that the differences observed between bacteria incubated with naïve and immune IgG antibodies is statistically significant using a paired *t* test.

Supplemental Fig S7. Vaccination with the *batA* KO mutant strain and passive transfer of immune serum provide protective immunity against challenge with lethal aerosol doses of WT organisms in C57BL/6 mice. Naïve C57BL/6 mice were administered 1-mL of immune or naïve serum intraperitoneally and, 48 hours later, challenged with $10LD_{50}$ of WT *B. mallei* ATCC 23344 or $4LD_{50}$ of WT *B. pseudomallei* strain 1026b; animals vaccinated with 10^4 CFU of the *B. mallei batA* KO mutant and back-challenged with WT organisms (30 days post-vaccination) were also tested. Vaccination with the *batA* KO mutant and challenge with WT bacteria were performed intratracheally using a Microsprayer device. Panel A: Kaplan-Meier survival curves. Panel B: Survival data during the acute and chronic phases of infection. Panels D, E, and F: Tissues were collected from mice that survived challenge (day 35), homogenized, diluted, and spread on agar plates to determine bacterial loads. Symbols show data for individual animals; horizontal bars represent the mean total CFU for each group.

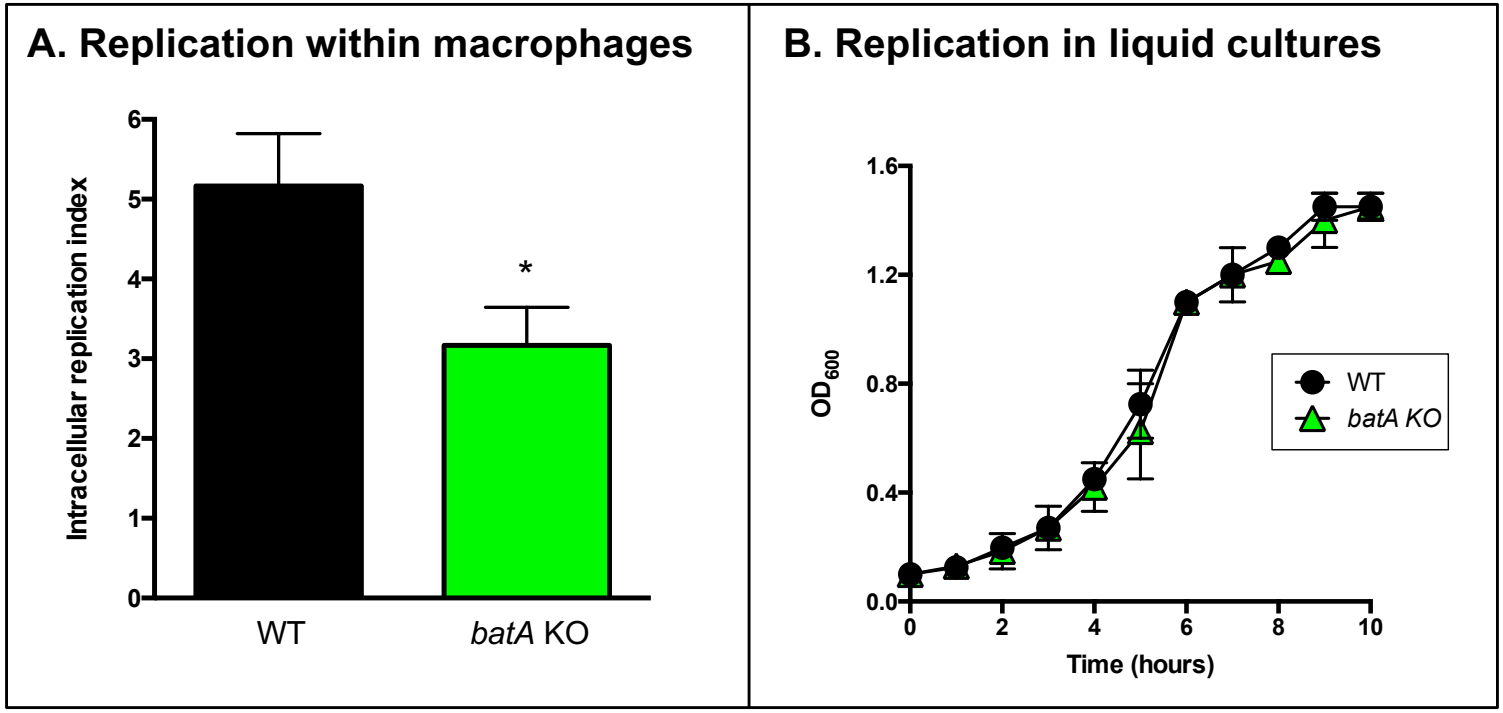
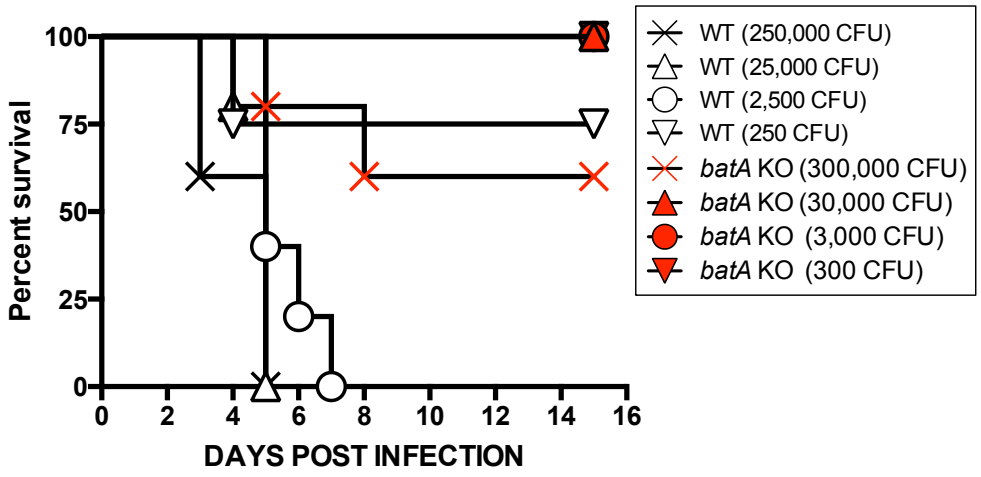


Fig S1. *In vitro* replication rates of *B. mallei* WT and mutant strains.

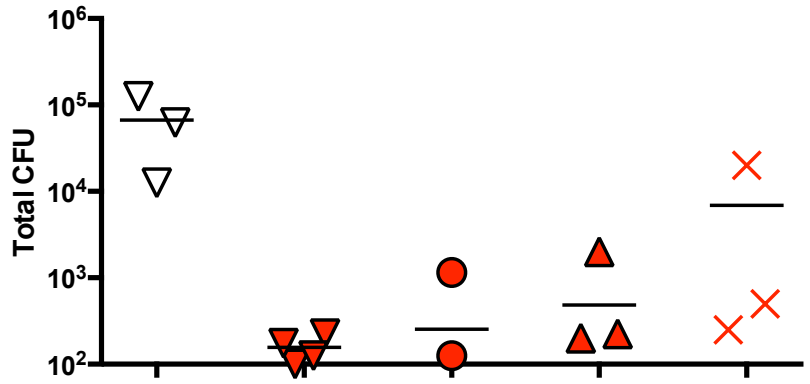
A. Survival curves



B. Median lethal doses (LD_{50})

Strain	Inoculum (CFU)	% survival	LD50 (CFU)
WT	250	60	370
	2,500	0	
	25,000	0	
	250,000	0	
batA KO	300	100	>440,000
	3,000	100	
	30,000	100	
	300,000	60	

C. Lung burden



D. Spleen burden

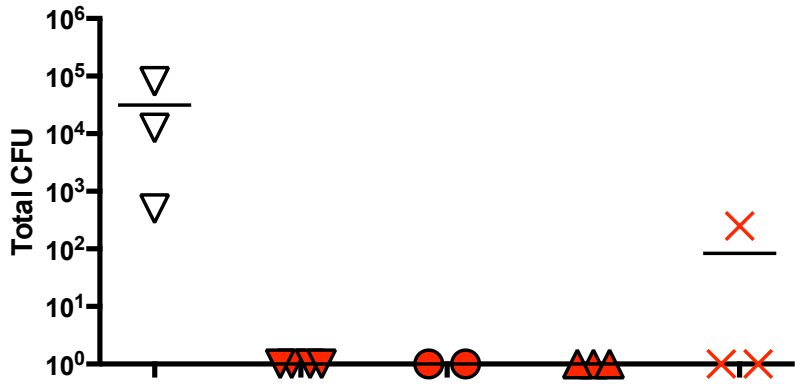
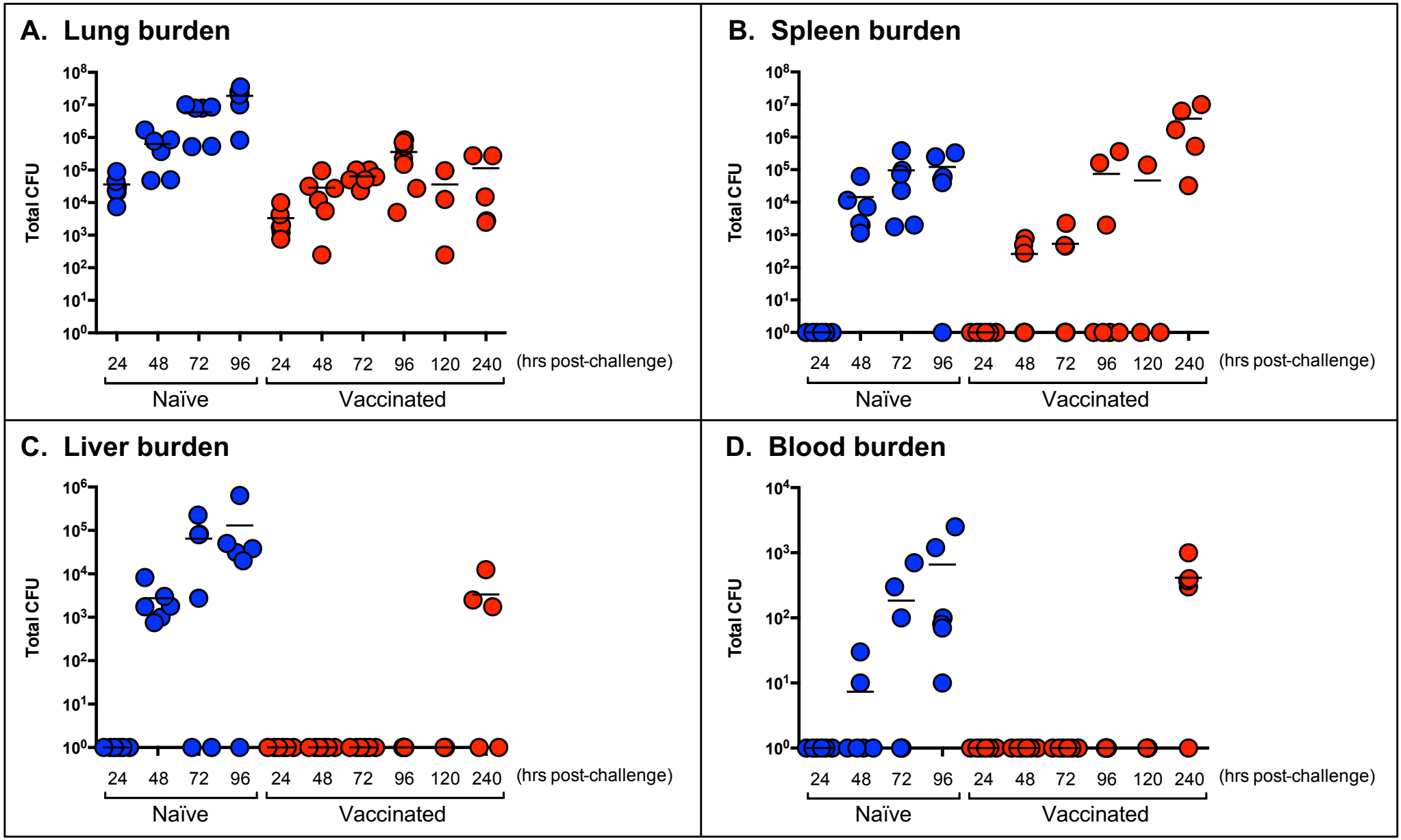


Fig S2. Median lethal dose comparison of *B. mallei* WT and mutant strains.



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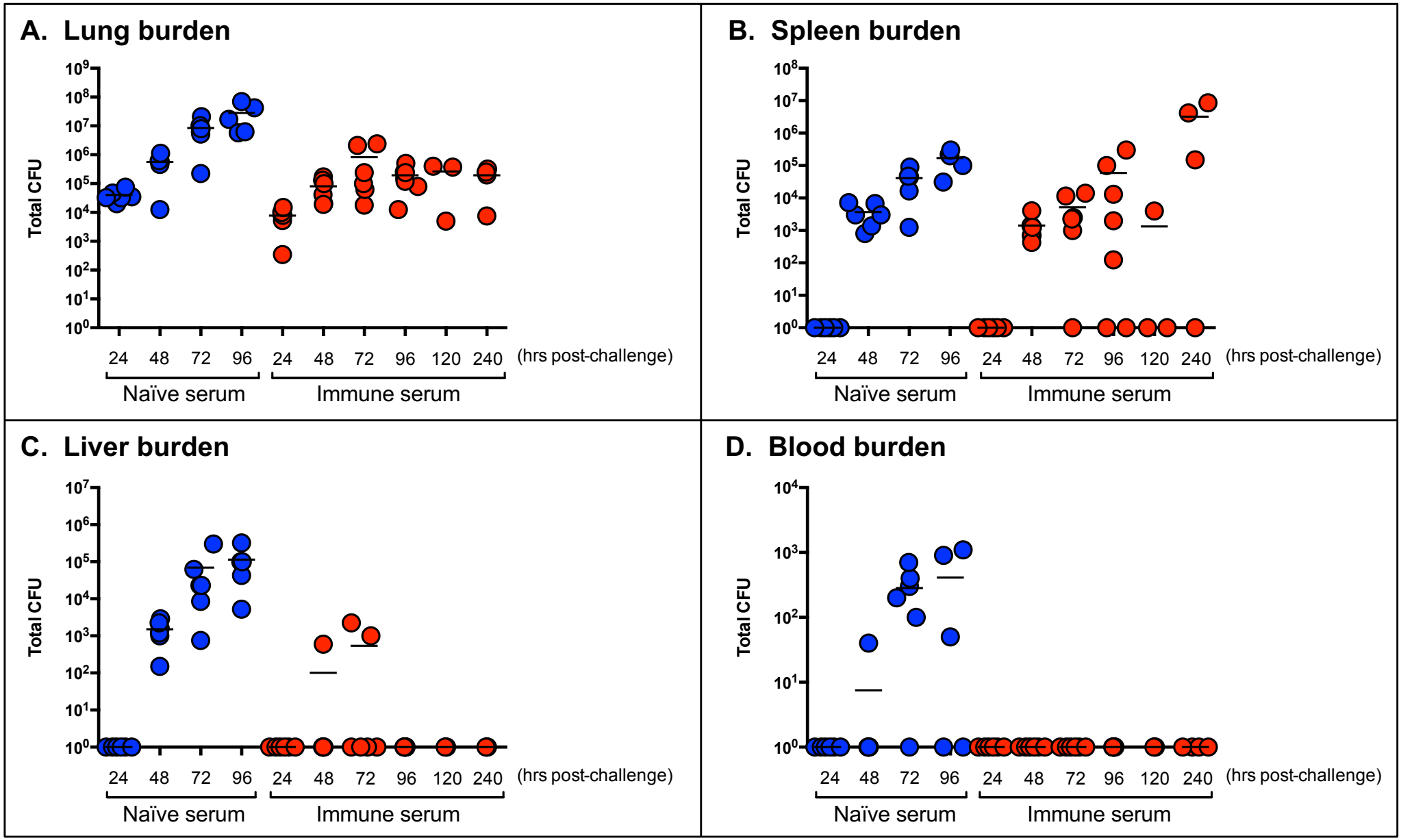


Fig S4. Kinetics of bacterial accumulation in target tissues of mice that were administered immune serum (from mice vaccinated with the *batA* KO strain).

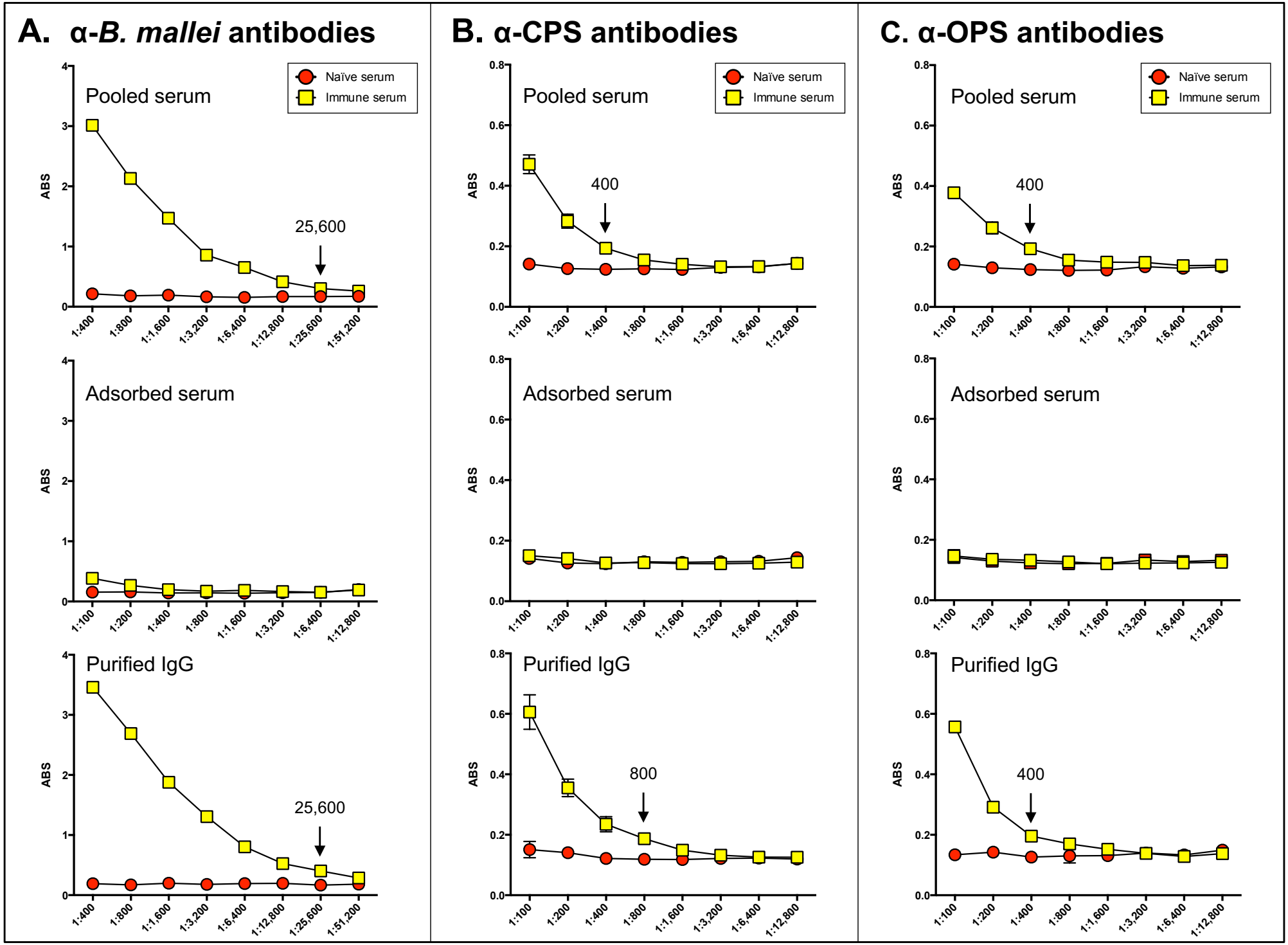


Fig S5. Reactivity of immune serum fractions with whole bacteria, capsular polysaccharides (CPS) and oligosaccharide chains of LPS (OPS).

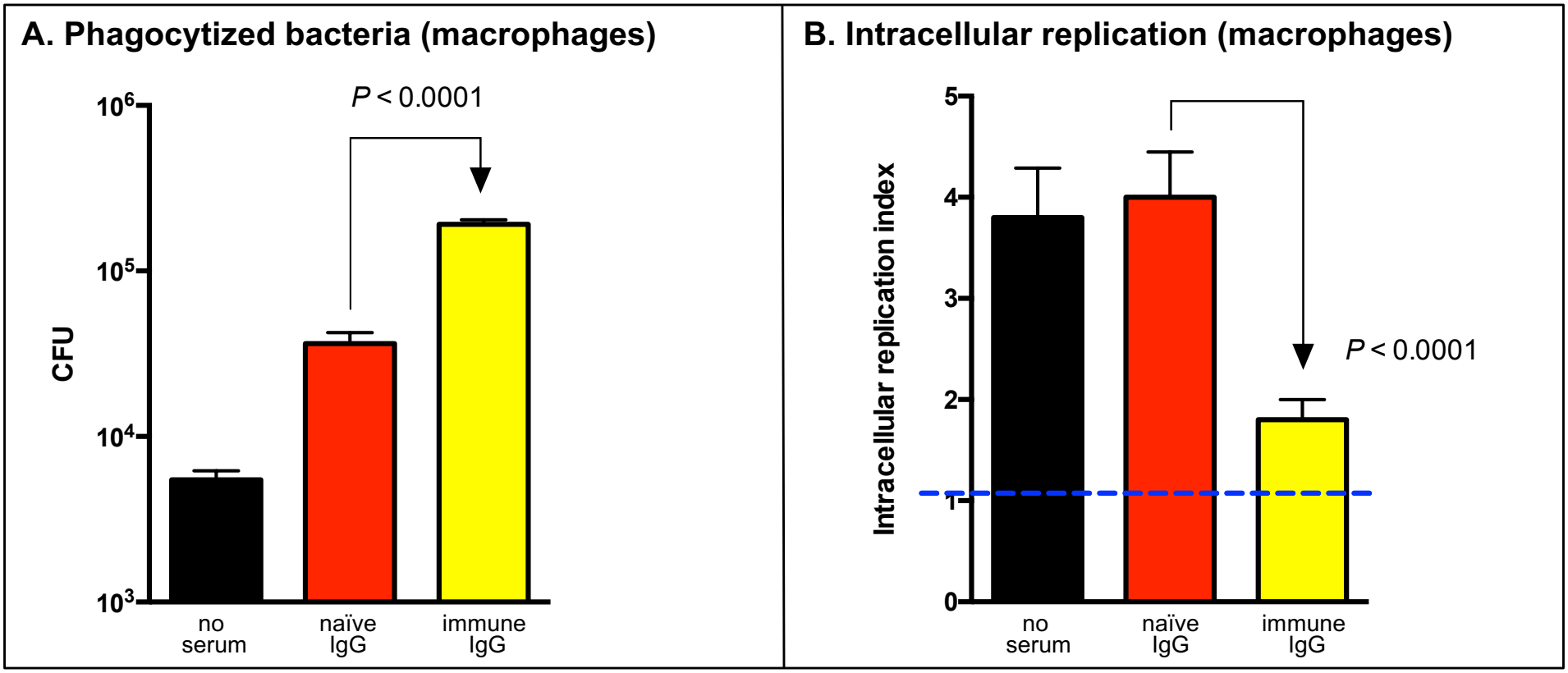
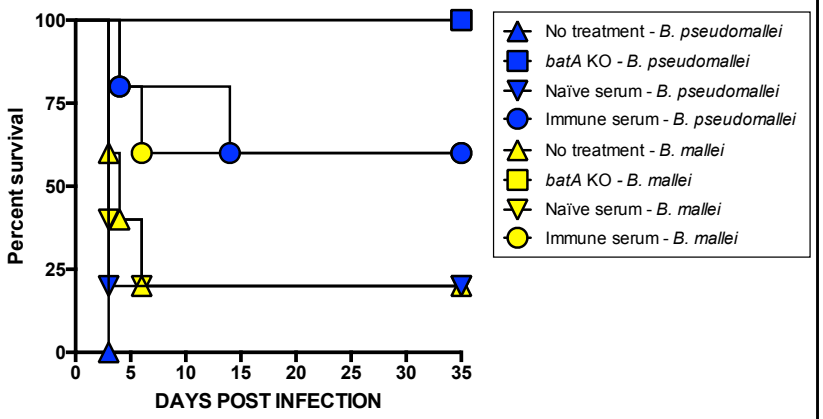


Fig S6. Opsonophagocytic killing assays using WT *B. mallei* and purified IgG antibodies.

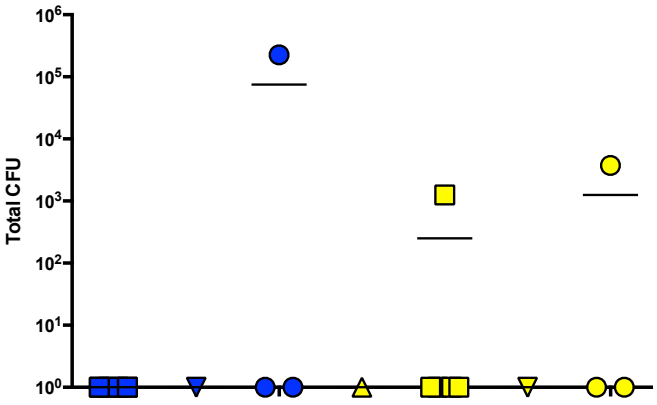
A. Survival curves



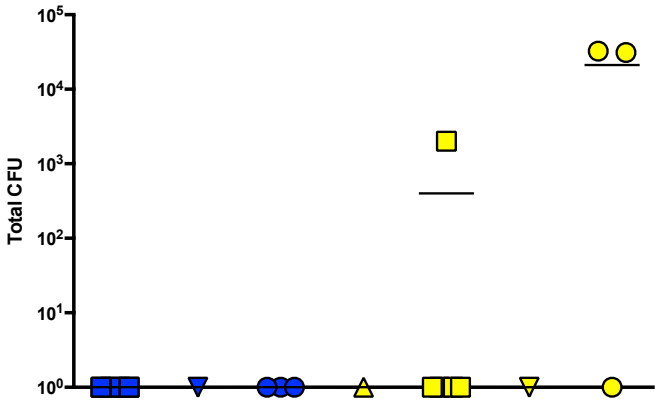
B. Survival data

Treatment	WT agent used in challenge	# mice challenged	Survival, acute infection (1-10 days post-challenge)	Survival, chronic infection (11-35 days post-challenge)
None	*B.pseudomallei* 1026b	5	0%	0%
batA KO vaccination		6	100%	100% (100% sterile)
Naïve serum		5	20%	20% (100% sterile)
Immune serum		5	80%	60% (67% sterile)
None	*B.mallei* ATCC 23344	5	20%	20% (0% sterile)
batA KO vaccination		5	100%	100% (60% sterile)
Naïve serum		5	20%	20% (0% sterile)
Immune serum		5	60%	60% (33% sterile)

C. Lung burden



D. Liver burden



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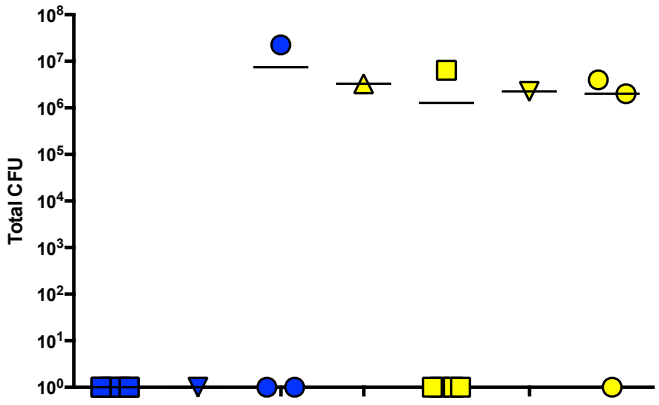

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