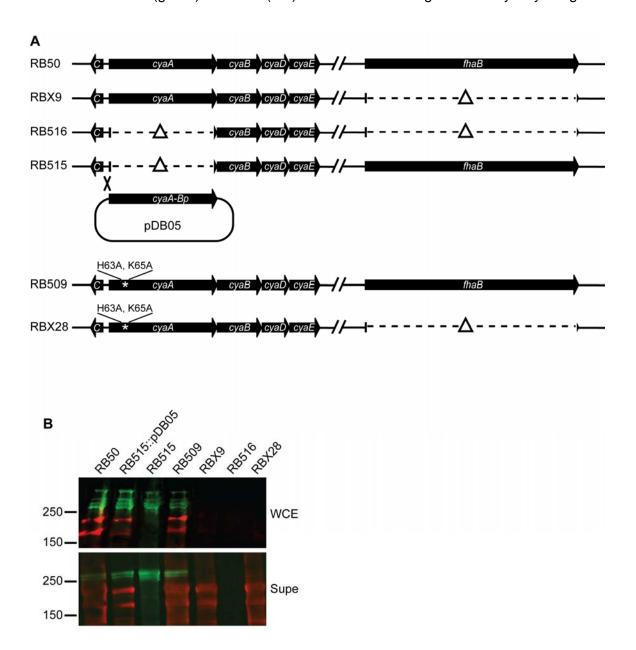
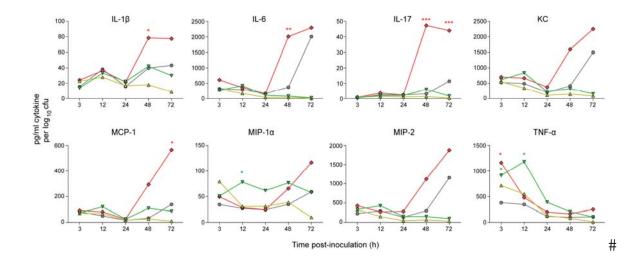
## Supplemental Figure 1. Protein production by mutant strains

**A**. Schematic depicting the *cyaA* and *fhaB* genetic loci for strains used in this study. **B**. Bacterial cultures were normalized based on optical density, whole cell lysates and culture supernatants were collected for SDS-PAGE protein separation and Western blot. Production of FHA (green) and ACT (red) were detected using a Licor Odyssey imager.



## Supplemental Figure 2. Cytokine production in lung homogenates normalized to the number of cfu

Lung homogenates assayed via Luminex technology for cytokine production (Fig. 3C) were further analyzed and normalized to a value per cfu of bacteria isolated at the given time point. Statistics are calculated as significant variation from wild type (\*=P < 0.05, \*\*=P < 0.001, \*\*\*=P < 0.0001).



rpe <i>B. bronchiseptica</i> expressing CyaA <sub>Bp</sub> instead of CyaA <sub>Bb</sub> AcyaA strain, contains only the first 4 and last 5 codons of expressing catalytically inactive CyaA	(1) This study This study
expressing CyaA <sub>Bp</sub> instead of CyaA <sub>Bb</sub> AcyaA strain, contains only the first 4 and last 5 codons of	This study
AcyaA strain, contains only the first 4 and last 5 codons of	
	This study
expressing catalytically inactive CyaA	
	This study
<i>IfhaB</i> strain, contains only the first 4 and last 5 codons of	(2)
∆ <i>fhaB∆cyaA</i> strain	This study
AfhaB strain expressing catalytically inactive CyaA	This study
AfhaS strain containing a 28 a.a deletion within the mature C- al domain of <i>fhaB</i>	(3)
rivative of clinical <i>B. pertussis</i> isolate Tohama I	(4)
ular cloning strain	BRL; Gaithersburg, MD
gation strain (diaminopimelic acid auxotroph)	(5)
tella suicide plasmid (Gent' Amp')	(6)
containing cyaA from B. pertussis strain BPSM	This study
exchange plasmid (Gent <sup>r</sup> Amp <sup>r</sup> Sucrose <sup>®</sup> )	(7)
S containing a large in-frame deletion of <i>cyaA</i> <sub>Bb</sub> , used to ate strains RB515 and RB516	This study
s H63A and K65A), used to generate strains RB509 and	This study
S containing a large in-frame deletion of the Boursed to	(7)
	ate strains RB515 and RB516 S encoding catalytically inactive CyaA ( <i>cyaA</i> <sub>Bb</sub> has mutated s H63A and K65A), used to generate strains RB509 and 8 S containing a large in-frame deletion of <i>fhaB</i> <sub>Bb</sub> , used to ate strains RBX9 and RB516

## Supplemental Table 1. Strains and plasmids used in this study

1. Cotter PA, Miller JF (1994) BvgAS-mediated signal transduction: analysis of phase-locked regulatory mutants of *Bordetella bronchiseptica* in a rabbit model. Infect Immun 62: 3381-3390.

2. Cotter PA, Yuk MH, Mattoo S, Akerley BJ, Boschwitz J, et al. (1998) Filamentous hemagglutinin of *Bordetella bronchiseptica* is required for efficient establishment of tracheal colonization. Infect Immun 66: 5921-5929.

3. Julio SM, Inatsuka CS, Mazar J, Dieterich C, Relman DA, Cotter PA. (2009) Natural-host animal models indicate functional interchangeability between the filamentous hemagglutinins of *Bordetella pertussis* and *Bordetella bronchispetica* and reveal a role for the mature C-terminal domain, but not the RGD motif, during infection. Mol. Microbiol 71: 1574-1590.

4. Menozzi, F. D., P. E. Boucher, G. Riveau, C. Gantiez, and C. Locht. 1994. Surface-associated filamentous hemagglutinin induces autoagglutination of *Bordetella pertussis*. Infect. Immun. 62:4261-4269.

5. Lopez, C. M., Rholl, D. A., Trunck, L. A., Schweizer, H. P. 2009. Versatile dual-technology system for markerless allele replacement in *Burkholderia pseudomallei*. Appl Environ Microbiol. 20:6496-503

6. Martinez de Tejada, G., J. F. Miller, and P. A. Cotter. 1996. Comparative analysis of the virulence control systems of *Bordetella pertussis* and *Bordetella bronchiseptica*. Mol. Microbiol. 22:895-908.

7. Cotter PA, Yuk MH, Mattoo S, Akerley BJ, Boschwitz J, et al. (1998) Filamentous hemagglutinin of *Bordetella bronchiseptica* is required for efficient establishment of tracheal colonization. Infect Immun 66: 5921-5929.