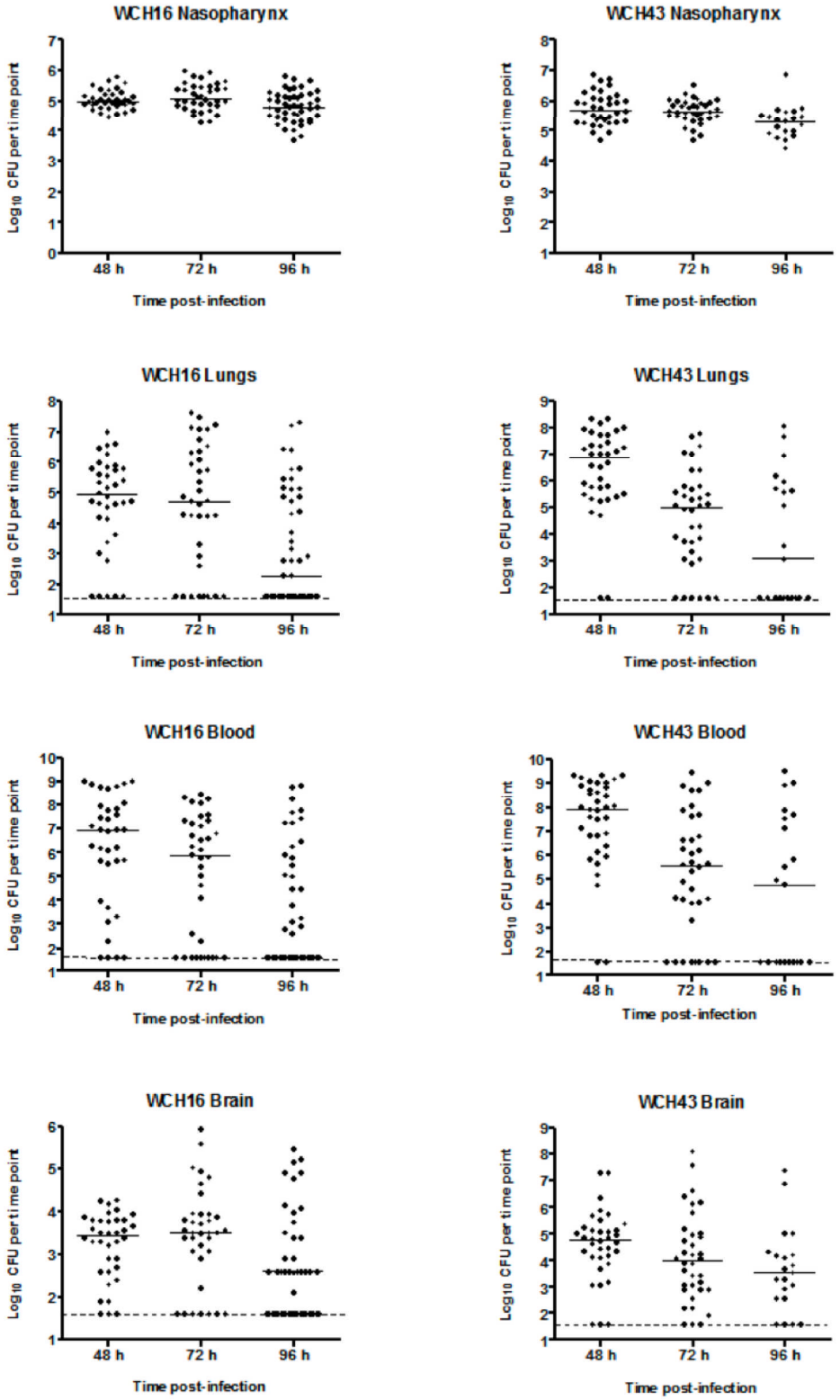
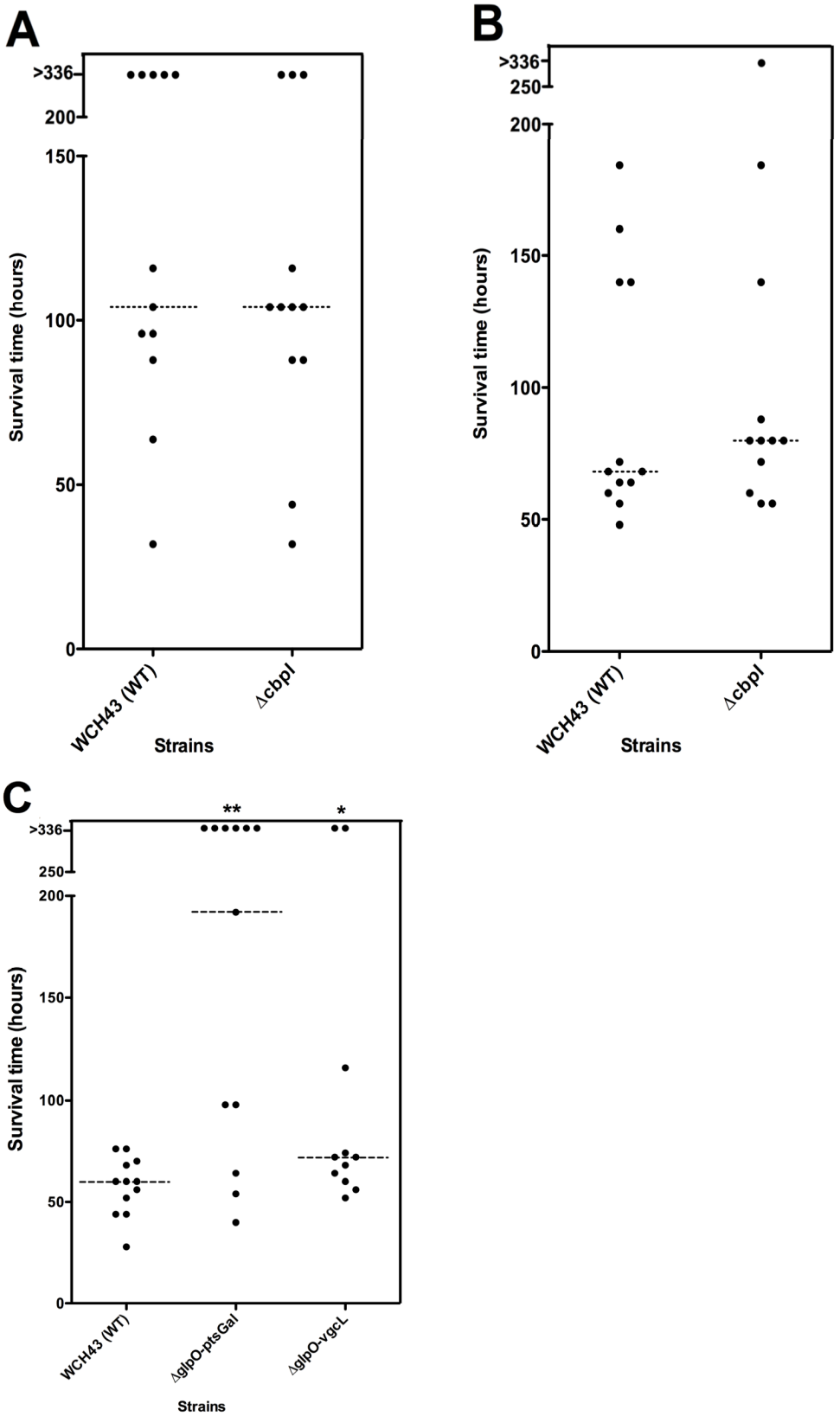


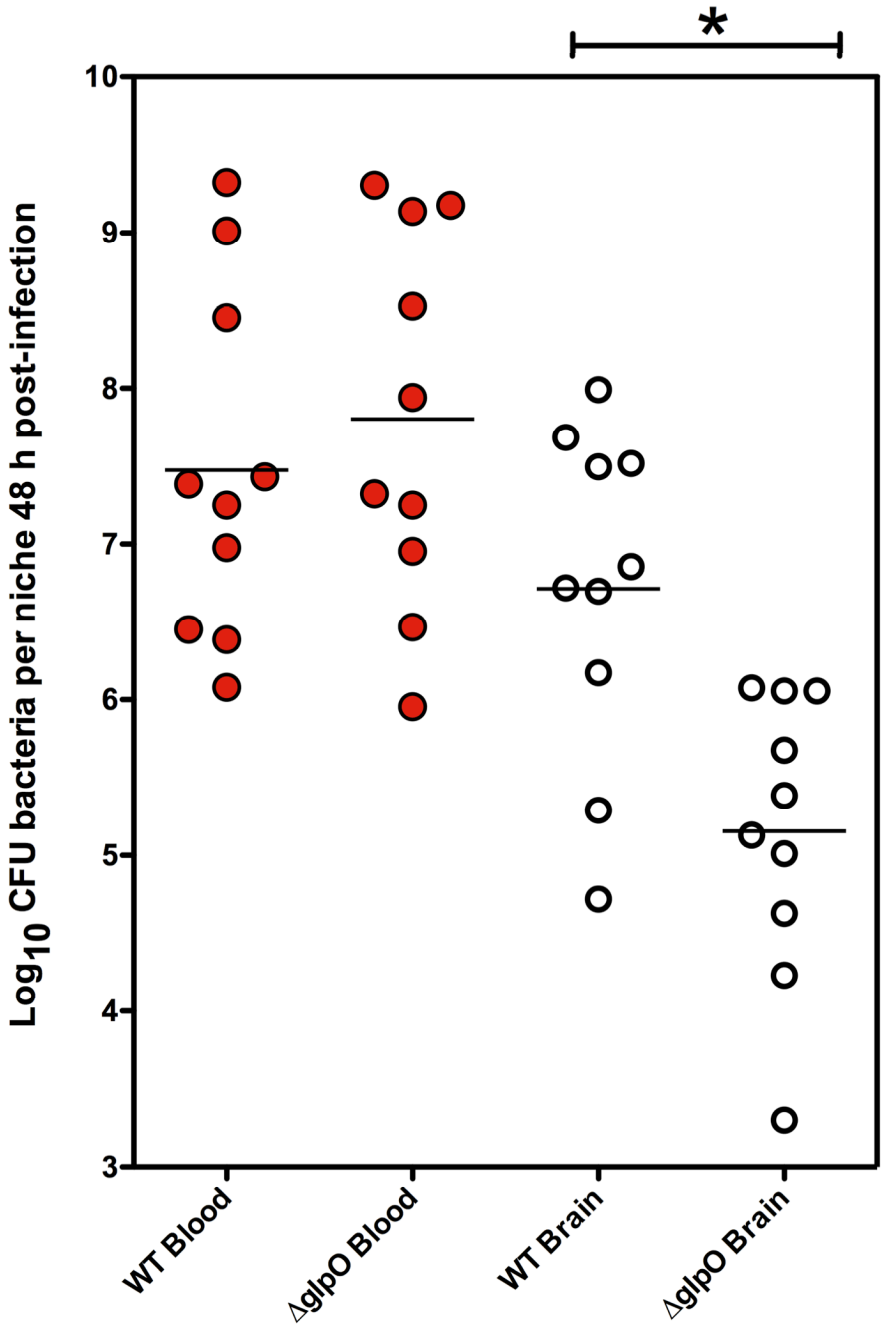
Supplemental Figure 1

Pathogenesis of pneumococcal meningitis in WCH16 and WCH43. Bacteria in the indicated niches of CD1 mice were enumerated at 48, 72, a nd 96 h after i.n. challenge. Each datum point represents one mouse. The horizontal lines indicate the median CFU recovered from each niche at each time point for the indicated strains. The limit of detection, indicated by dashed lines, was 40 CFU for all niches.

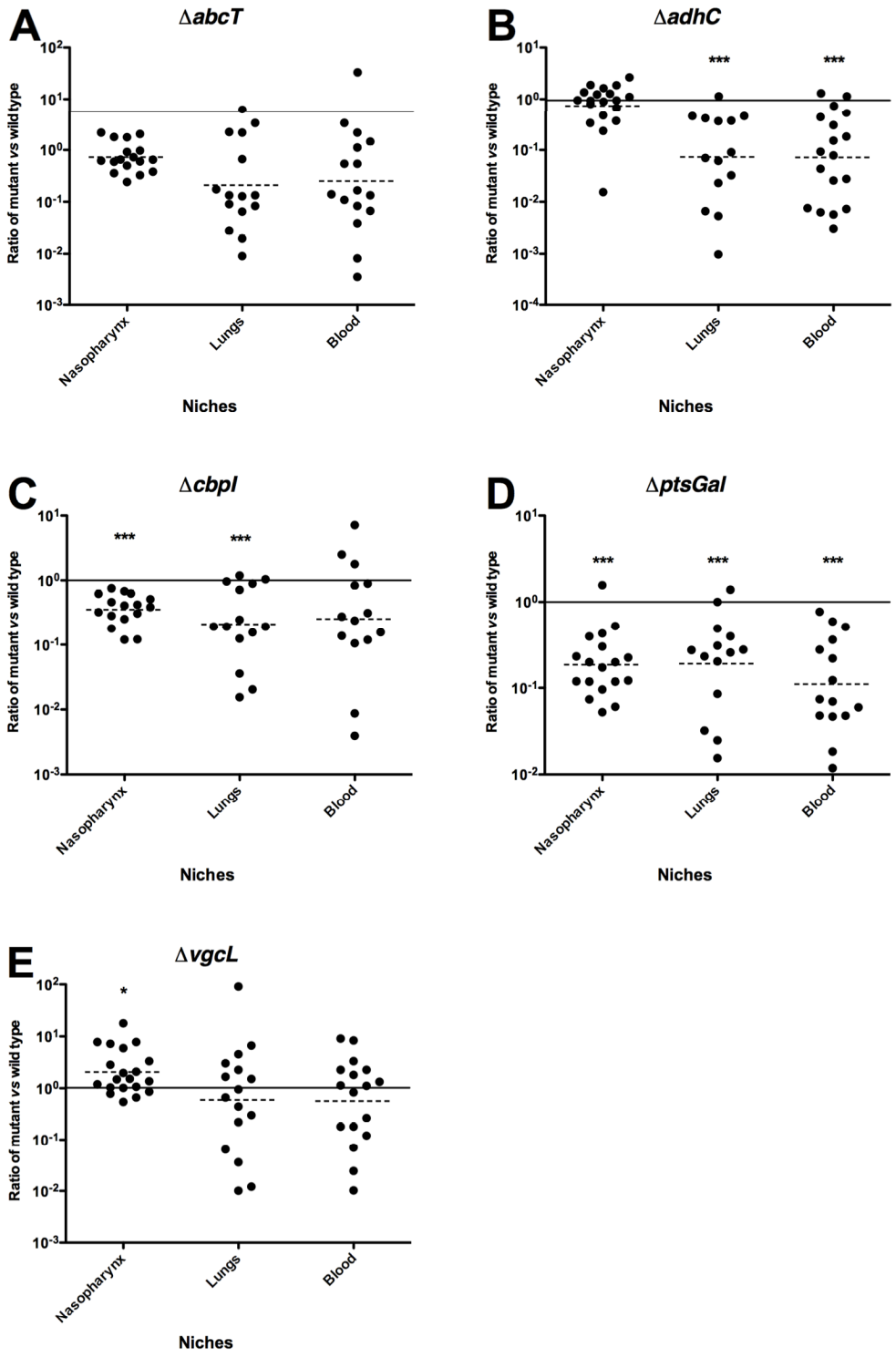


Supplemental Figure 2

Characterization of single Δ*cbpI* and double Δ*glpO* + Δ*ptsGal* and Δ*glpO* + Δ*vgcL* mutants of *S. pneumoniae* WCH43. Survival times for mice after (**A**) i.p., and (**B**) i.n. challenge with Δ*cbpI* mutant vs wild type. (**C**) Survival times after i.n. challenge with Δ*glpO* + Δ*ptsGal* and Δ*glpO* + Δ*vgcL* mutants vs wild type. Groups of 12 CD1 mice were challenged i.p. with approx. 1×10^5 CFU, and i.n. with approx. 2×10^7 CFU of wild type WCH43 and its isogenic mutant strains. Each datum point represents one mouse. The horizontal broken lines denote the median survival time for each group. * $P < 0.05$; ** $P < 0.01$; Mann Whitney *U*-test, one-tailed.

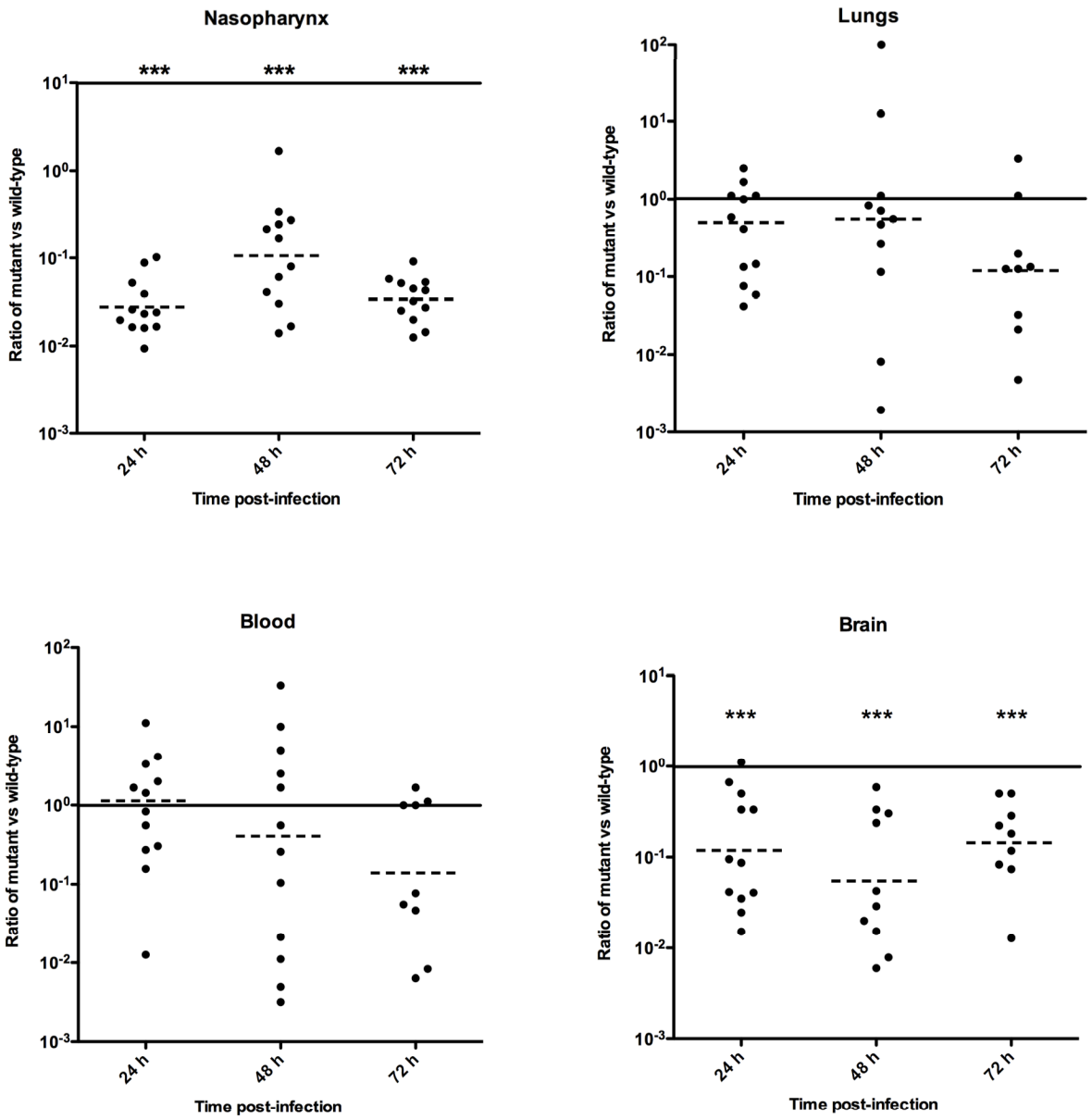


Supplemental Figure 3
Pathogenesis of WCH43 and its isogenic Δ*glpO* mutant. 10 CD1 mice were infected i.n. with either wild type or mutant, and bacteria in the blood and brain of each mouse were enumerated at 48 h post-infection. Horizontal line represents geometric mean. * $P < 0.05$; unpaired *t*-test, one-tailed.



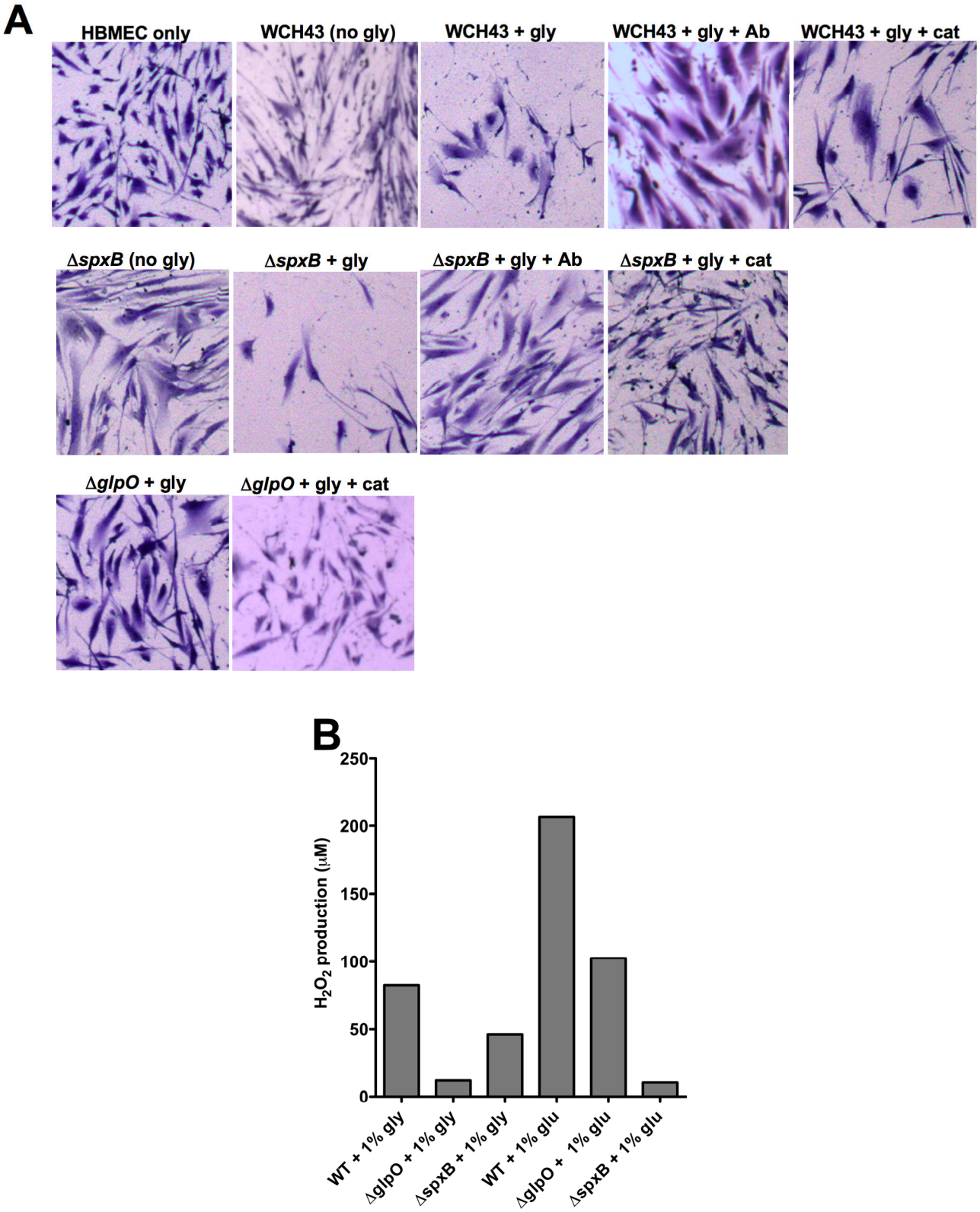
Supplemental Figure 4

Competition experiments between wild type WCH43 and isogenic mutants in the nasopharynx, lungs, and blood of mice at 48 post-infection. 14-20 CD1 mice were infected i.n. with equal numbers (approx. 1×10^7 CFU each) of wild type and mutant. Each datum point represents the ratio of mutant bacteria to wild type for each mouse. The horizontal solid line denotes a 1:1 ratio. The horizontal broken line denotes the geometric mean ratio of mutant:wild type for each comparison. * $P < 0.05$; *** $P<0.001$; one sample *t*-test, two-tailed.



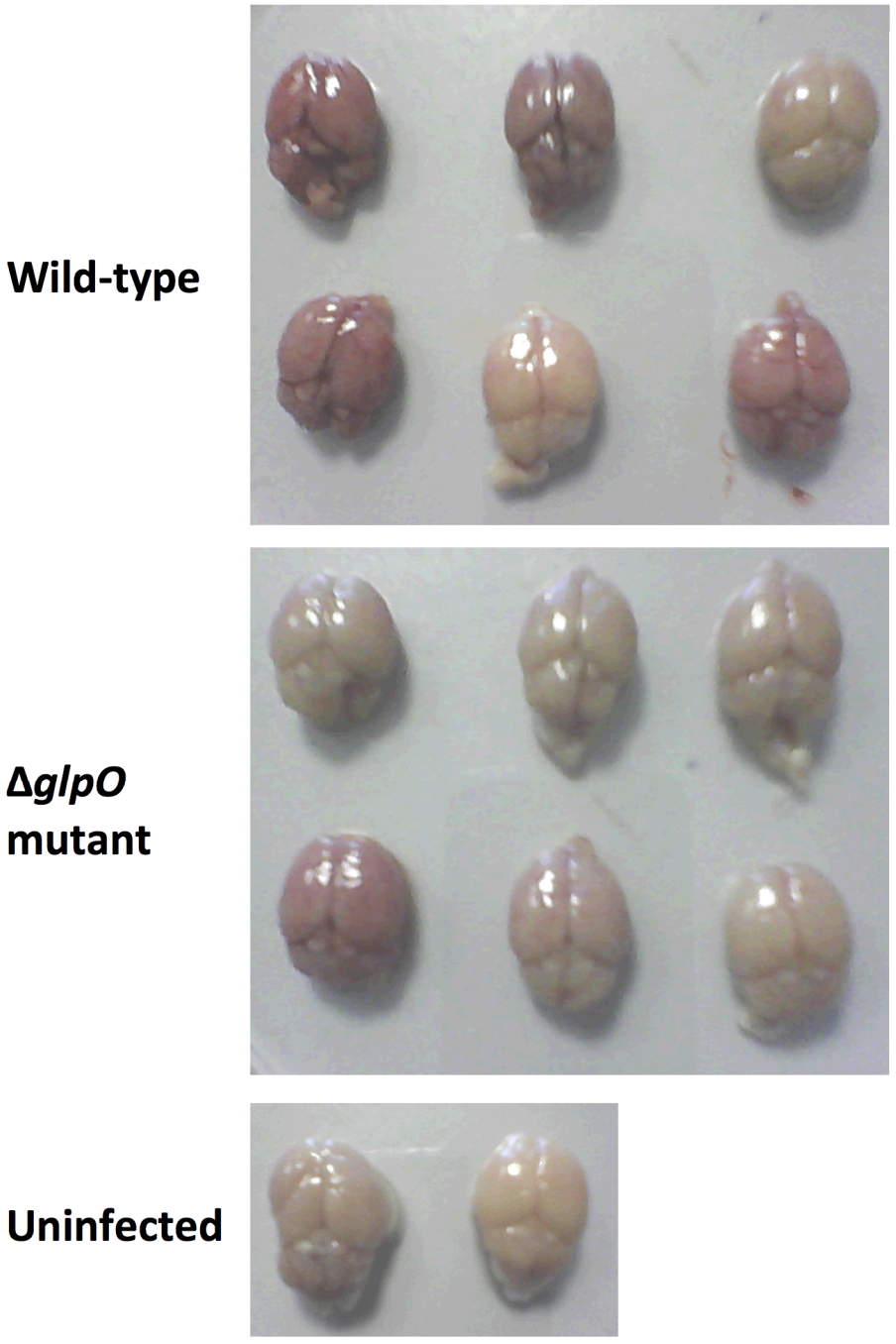
Supplemental Figure 5

Competition experiments between wild type WCH43 and its isogenic Δ*glpO* mutant in the nasopharynx, lungs, blood and brain of mice at 24, 48, and 72 h post-infection. 10-12 CD1 mice were infected i.n. with equal numbers (approx. 1×10^7 CFU each) of wild type and mutant. Each datum point represents the ratio of mutant:wild type for each mouse. The horizontal solid line denotes a 1:1 ratio. The horizontal broken line denotes the geometric mean ratio for each comparison. *** $P<0.001$; one sample *t*-test, two-tailed.



Supplemental Figure 6

HBMEC cytotoxicity and H_2O_2 production. (**A**) Crystal violet-stained HBMEC monolayers after infection with the strain and supplements, as indicated on top of each panel and detailed in the Methods. (**B**) H_2O_2 production. The indicated strains were cultured in medium supplemented with glycerol (gly) or glucose (glu) and assayed for H_2O_2 production as detailed in the Methods. WT = Wild type WCH43; cat = catalase; Ab = polyclonal anti-GlpO serum.



Supplemental Figure 7
Blood-brain permeability. Mice were infected with WCH43 or Δ*glpO* and after 48 h, injected i.p. with Evans Blue. After a further 2 h, mice were euthanased and perfused as described in the Methods. Brains were then removed and photographed.

Table S1. *S. pneumoniae* strains used in this study.

Strain	Description (Sequence type)	Source/Reference
D39	Capsular serotype 2 (595)	NCTC 7466
D39::Δ*fhs*	*fhs* deletion mutant of D39 [Spec^R]	Present study
WCH16	Capsular serotype 6A clinical isolate (4966)	Women's and Children's Hospital, North Adelaide, Australia
WCH16::Δ*fhs*	*fhs* deletion mutant of WCH16 [Spec^R]	Present study
WCH43	Capsular serotype 4 clinical isolate (205)	Women's and Children's Hospital, North Adelaide, Australia
WCH37	Capsular serotype 33 clinical isolate	Women's and Children's Hospital, North Adelaide, Australia
WCH163	Capsular serotype 35F clinical isolate	Women's and Children's Hospital, North Adelaide, Australia
Δ*abcT*	*abcT* deletion mutant of WCH43 [Ery^R]	Present study
Δ*adhC*	*adhC* deletion mutant of WCH43 [Ery^R]	Present study
Δ*cbpI*	*cbpI* deletion mutant of WCH43 [Ery^R]	Present study
Δ*fhs*	*fhs* deletion mutant of WCH43 [Spec^R]	Present study
Δ*glpO*	*glpO* deletion mutant of WCH43 [Spec^R]	Present study
Δ*ptsGal*	*ptsGal* deletion mutant of WCH43 [Spec^R]	Present study

Δ*spxB*	*spxB* deletion mutant of WCH43 [Ery^R]	Present study
Δ*vgcL*	*vgcL* deletion mutant of WCH43 [Spec^R]	Present study
Δ*glpO* + Δ*ptsGal*	Double *glpO* and *ptsGal* deletion mutants of WCH43 [Ery^R+ Spec^R, respectively]	Present study
Δ*glpO* + Δ*vgcL*	Double *glpO* and *vgcL* deletion mutants of WCH43 [Ery^R+ Spec^R, respectively]	Present study

Table S2. Primers used in this study.

Primers for real time RT-PCR[A]	Sequence (5'→ 3')
16S F	AAGCAACGCGAAGAACCTT
16S R	GTCTCGCTAGAGTGCCCAAC
abcT F	TTTGTGATGAACCGACTGGA
abcT R	GCACCACATCCTTGACACTG
adhC F	TGTTCAAAAAGGGGACAAGG
adhC R	CACGCACCTTGGTAATTCCT
cbpA F	GTGTTGGAGTAGCTAGTGTAGTTG
cbpA R	TCAACTATTTTATTCAAATACTCGTTC
cbpI F	ATGGTTCTACTTTGACGGCTCAGGAGC
cbpI R	TACCCATTCACCATTACCATTTACACG
fhs F	CTGTTGGTCTTGGTGGTCCT
fhs R	ACCCTCAACCTGCAAATCAC
glpO F	TCCATCTGCAGTTTCTCGTG
glpO R	TTTGCTGGGTTCAATTCTCC

ply F	GATGGCAAATAAAGCAGTAAATGACT
ply R	GCTACTTGCCAAACCAGGCAAATC
prtA F	GTTGGAGAGGCCAAAAACAA
prtA R	CCATGTTCTCCACCAATTCC
pspA F	CAAGTCTAGCCAGCGTCGCTATCT
pspA R	GAACAACAAGCGCCACATCATTC
ptsGal F	TGATTCCTCACACAGATATTGTTCA
ptsGal R	CCATTTCCTGTGAAGAAGTCCAT
sodA F	GCATTTGCACCATGACAAAC
sodA R	AGGCTGCTTGGAATTCTTCA
spxB F	CAACATGTGCTACCCAGACG
spxB R	CGAGCATCGATGACAACAGT
vgcL F	ATTGAAAAGGGCTTCCACCT
vgcL R	ACCAGTTGAGGGACATGAGG

Primers for constructing mutants[A]	**Sequence (5'→ 3')**

abcT Flank F	CGGACTATCACTTAAATGTCAATCT
abcT Ery F	TTGTTCATGTAATCACTCCTTCATCAAACAAATTATACTCCTCGAAAATC
abcT Flank R	TGAAGTTCCCTCCAAGCCACCAG
abcT Ery R	CGGGAGGAAATAATTCTATGAGGCGAAAAACTTATTGGAAGGACTTA
adhC Flank F	GAATGAGAAATATGAAGGCAAAATATG
adhC Ery F	TTGTTCATGTAATCACTCCTTCTCATTTCATTCACTGAATTCACGCT
adhC Flank R	TGCTCTTTTTCAAACTTGAGGTC
adhC Ery R	CGGGAGGAAATAATTCTATGAGAGGAGTTTTAGAACTCTATTCGTTTT
cbpI Flank F	GCGGGTCGTATTGCAGGTGCG
cbpI Ery F	TTGTTCATGTAATCACTCCTTCGGTTAATTAGCGACTCATAGTGAC
cbpI Flank R	CGGGTCCTTGTGTACCTTTGGT
cbpI Ery R	CGGGAGGAAATAATTCTATGAGATAGGTAAAGCATTCATCTTACTTAG
glpO Flank F	TGGCTGAAAACGGTGGCCAGC
glpO Spec X	TATGTATTCATATATATCCTCCTCCTAAATAAATCGCTTTATTCTGCCAG
glpO Flank R	GTCTAGCATTTCCCCGTCGCTTG
glpO Spec Y	AAATAACAGATTGAAGAAGGTATAAGAAAAAATAAAAGAGGTGGAGGGCA
fhs Flank F	CAATGGCTAGGGTTAGAATTGCAG

fhs Spec X	TATGTATTCATATATATCCTCCTCAACCTCTTATATGATAATTCATTATATC
fhs Flank R	TCTACTGGCATAGGTTGAGAACG
fhs Spec Y	AAATAACAGATTGAAGAAGGTATAATGGATTGAAATTGACTTTAAGTGAGT
ptsGal Flank F	CTTCTCAATGGAGATAAGGTGGAT
ptsGal Spec X	TATGTATTCATATATATCCTCCTCTCAAAAAGTTATTCTTGATTATCCGC
ptsGal Flank R	ATGGTTATAGGCTTTATCTTCAACTG
ptsGal Spec Y	AAATAACAGATTGAAGAAGGTATAATAGTTCTATGAATGATGAAGCAAGTA
spxB Flank F	GTTGCAGGTAAGCCATATATCCAG
spxB Ery X	TTGTTCATGTAATCACTCCTTCTTCAATTTTTTTAAACTTGGAGAATACG
spxB Flank R	AACCCCGTCTTTGTAAATGGCATC
spxB Ery R	CGGGAGGAAATAATTCTATGAGCCGAAAATCAAATATGAAACTTGTAG
vgcL Flank F	GACCAAGATTGTTGGTGATAAACCA
vgcL Spec X	TATGTATTCATATATATCCTCCTCAATTAACTTTCTTTTTTACCTAAAACCAG
vgcL Flank R	TGGGGTGTTACACGCACTTTAAAAT
vgcL Spec Y	AAATAACAGATTGAAGAAGGTATAATATGAGTATTTACATAATTTATGTTATGTA

Primers for	**Sequence (5'→ 3')**

cloning[A]	
glpO F	TTAAAAAAATGCATGCACGTACCCTGG
glpO ΔFADF	GTGTAGCCTTGCATGCGGCAGCTAGCG
glpO R	CTGTCTCCATTAAAAAGAAGGGACGGTCGACAAGGAATGC

[A] Primers were derived from the *S. pneumoniae* TIGR4 (serotype 4) genome as deposited in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.