Supplementary Information for

Recognition of Conserved Antigens by Th17 Cells Provides Broad Protection against Pulmonary *Haemophilus Influenzae* Infection

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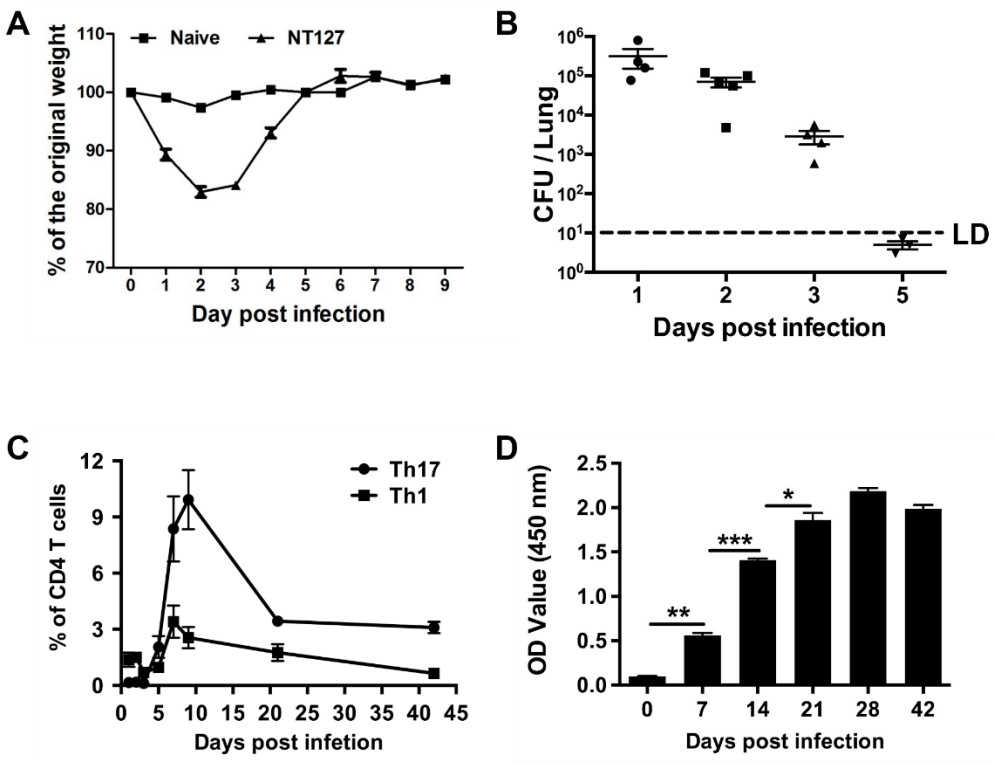

Fig. S1. Kinetics of immune response to NT127 infection. B6 mice were infected with NT127. Body weight change (A) and bacteria load in the lung (B) were measured. Kinetics of NT127-specific CD4 T cells producing IFN-γ (Th1) and IL-17 (Th17) (C), and antibody responses (D) were determined. n= 3-4, error bars = means ± SEM. *** $P < 0.001$;** $P < 0.01$; * $P < 0.05$. LD, limit of detection.

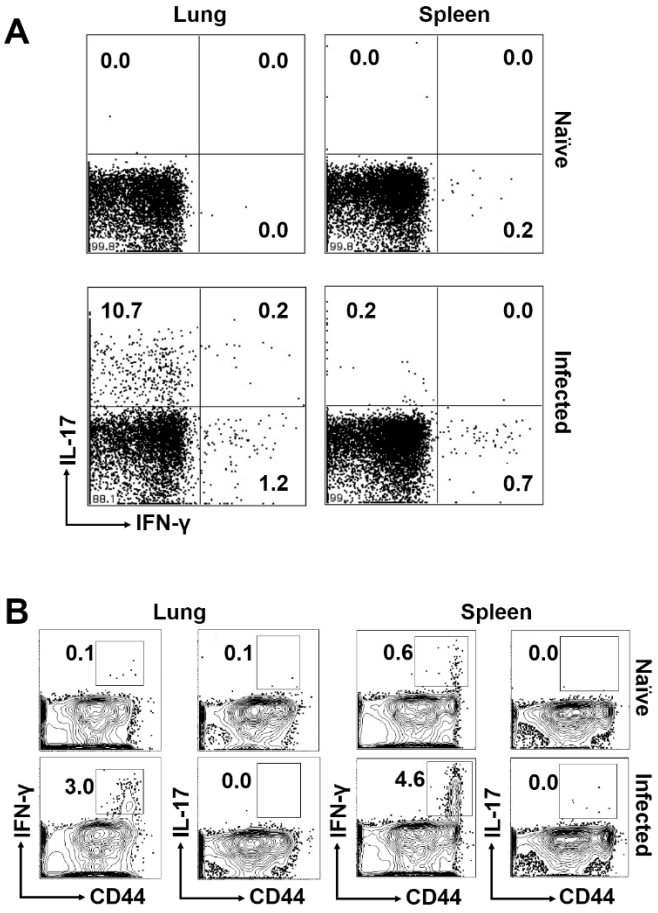

Fig. S2. Production of cytokines by T cells after NT127 infection. Lymphocytes from the lung and spleen of NT127-infected mice (day7) were stimulated with heat-killed NT127, followed by ICS for IFN-γ/IL-17 co-expression by CD4 T cells (A), and production of IFN-γ and IL-17 by CD8 T cells (B).

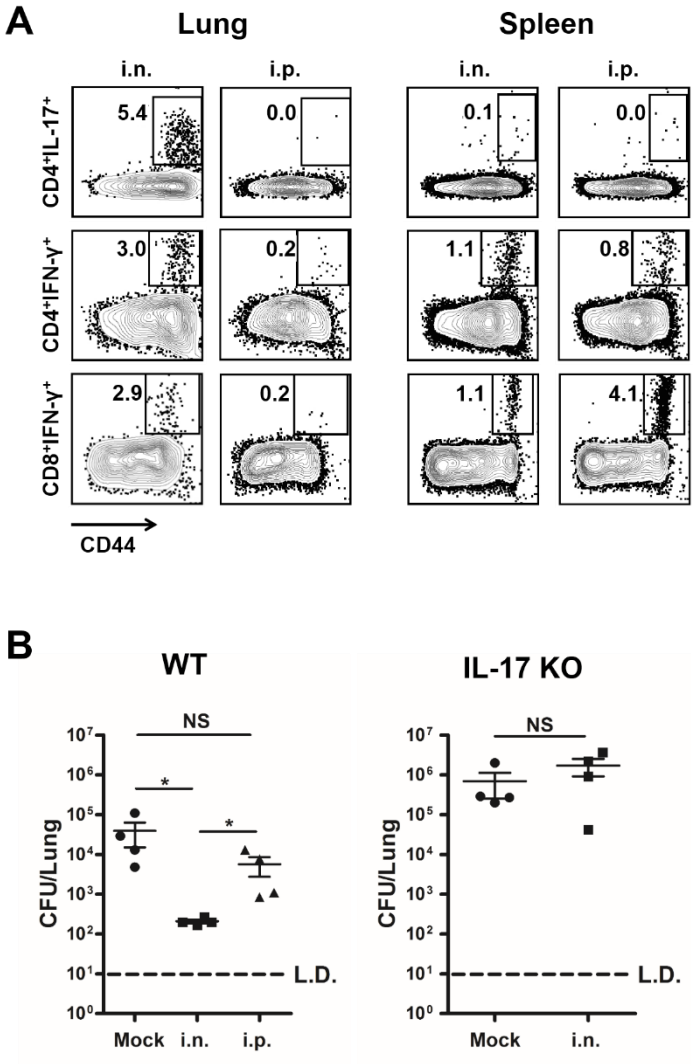

Fig. S3. IL-17 is important for heterologous protection. (A) B6 mice were immunized with NT127 via intranasal (i.n.) or intraperitoneal (i.p.) route. On D7 after infection, NT127 specific CD4 and CD8 T cell response in the lung and spleen were examined by ICS of IFN-γ and IL-17 following stimulation with heat-killed NT127. (B) B6 WT and IL-17 KO mice were immunized i.n. or i.p. with heat-killed NT127. Three weeks after immunization, immune mice and naïve controls were challenged intranasally with the heterologous strain 86-028NP. Two days after challenge, bacterial loads in the lung were determined. n=4, error bars = means ± SEM. **P* < 0.05; NS, not significant. L.D., limit of detection.

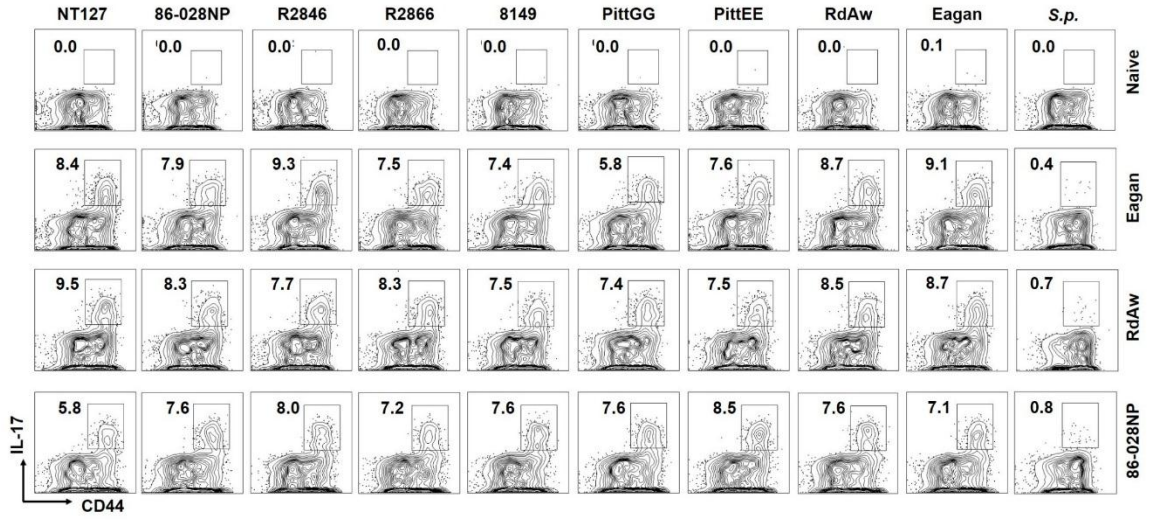

Fig. S4. Broad cross-reactivity of Th17 cells. Lung lymphocytes from naïve mice and mice infected with Eagan, RdAw or 86-28NP were stimulated with heat-killed bacteria of 9 different *H. influenzae* strains, and *S. pneumoniae* (*S.p.*). Activation of reactive Th17 cells was measured by ICS of IL-17 expression.

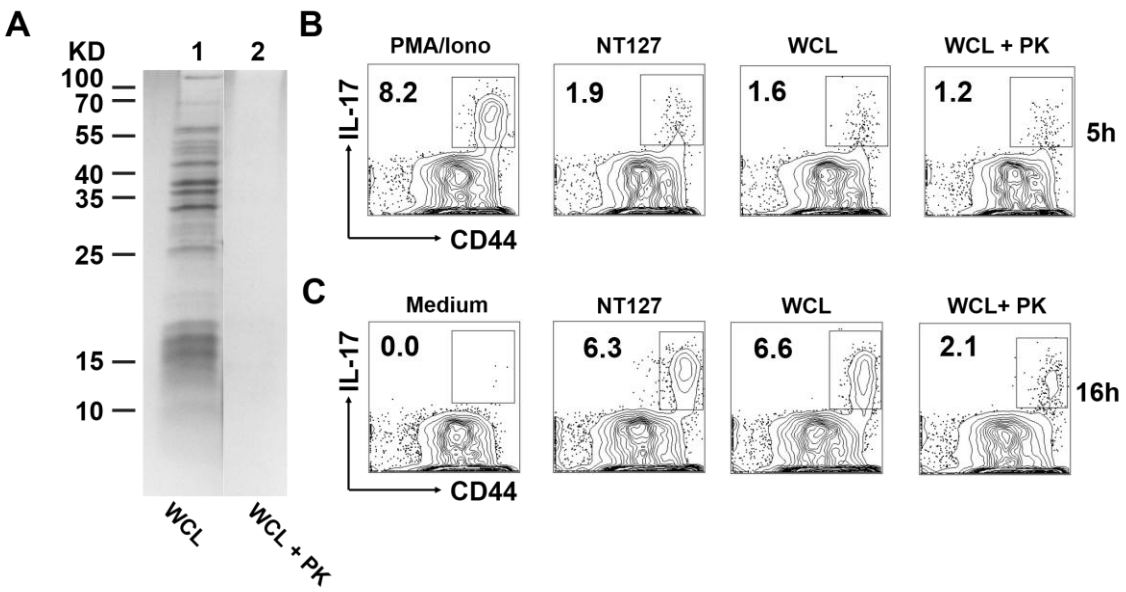

Fig. S5. Th17 response to NTHi is directed primarily to protein antigens. (A) Whole cell lysate of NT127 was treated with protease K (WCL+PK) or buffer alone (WCL) and analyzed by SDS-PAGE. (B) Lung cells from NT127 infected mice were pooled and stimulated with heat-killed NT127 or NT127 cell lysate treated or untreated with protease K for 5 h. PMA/ionomycin stimulation (PMA/Iono) was used as positive control. (C) Lung cells were also cultured with the indicated bacterial preparations for 16 h. Production of IL-17 by CD4 T cells was analyzed by ICS and representative data were shown.

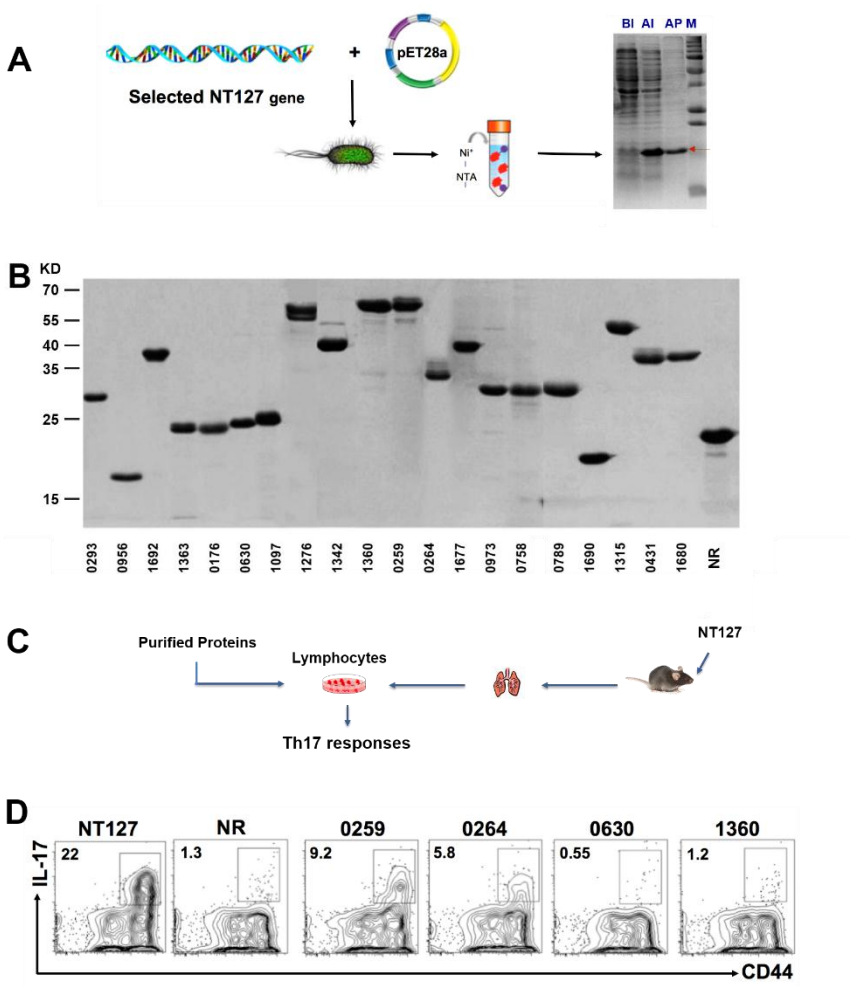

Fig. S6. Strategy for identification of Th17 antigens. (A) Genes encoding selected NTHi proteins were cloned into pET28a plasmid. Recombinant proteins were expressed in *E. coli* BL21 to high levels by IPTG induction and purified by Ni affinity column. The SDS gel image showed purification of a representative protein (BI, before induction; AI, after induction; AP, after purification; M, marker). (B) SDS gel images showing purity of 20 proteins purified with Ni column as described in the material and methods. (C) Purified individual proteins were used to simulate lung lymphocytes from NT127-infected mice, and IL-17 producing CD4 T cells were detected by ICS. (D) FACS plots of representative proteins that were identified as positive (0258 & 0264) and negative (0630 & 1360). Heat-killed NT127 and a non-relevant protein (NR, the negative control) were included as a positive and negative control, respectively.

Table S1. PCR Primers

No.	Gene No.	Pimer	Primer sequence (5'-3')
1	HIAG00259	Forward	GGG **CCA TGG** TGC AAC ACA AAC TAC TCT
		Reverse	GGG **CTC GAG** ATG TTT AAT AAT ATA AAG
2	HIAG00264	Forward	GGG **CCA TGG** TGA CAA CAA AAA CAA CT
		Reverse	CGG **CTC GAG** CAA TCC TAA CTT TTC TTC
3	HIAG01276-1	Forward	GGC **CCA TGG** TGC GAT TTT CTA AAC T
		Reverse	GGC **CTC GAG** CAC CCC ATA AAC AAA G
4	HIAG01276-2	Forward	GCG **CCA TGG** ATG GGG TGG ATT ATA T
		Reverse	GCG **CTC GAG** GAA GCT ATA AAC TGC ACT
5	HIAG00176	Forward	GGG **CCA TGG** TGA AAA ACA TCG CAA AAG T
		Reverse	GGG **CTC GAG** TTT TTT CTC TTG TGC T
6	HIAG00630	Forward	GGG **CCA TGG** TGA AAA AAA CAA CCT T
		Reverse	GGG **CTC GAG** TTT TTT ACG TTG ATC AT
7	HIAG01097	Forward	GGG **CCA TGG** TGC TCG CAA AAT TGT T
		Reverse	GGG **CTC GAG** AGT TTT ACC TTC AGC
8	HIAG01360	Forward	GGG **CTC GAG** AGT TTT ACC TTC AGC
		Reverse	GGG **GCG GCC** GCC TTC GCA ATA CGT TTA T
9	HIAG01677	Forward	GCG **CCA TGG** TGA AAA AAC TTT TAA AAA T
		Reverse	GCG **CTC GAG** TTT AGC TAA ACA TTC TAT G
		Reverse	GGC **CTC GAG** AAC ATT TTC TAC CGC CT
10	HIAG00758	Forward	CGC **CCA TGG** TGA ATA TCA CAG CCA T
		Reverse	GGC **CTC GAG** TTT ATC CTT ATT TTG AC
11	HIAG00293	Forward	CGC **CCA TGG** TGA TCG TCA ATT TTA T
		Reverse	GCC **CTC GAG** ACC TGC GCC AAA CAT AAT
12	HIAG01342	Forward	CCG **GCT AGC** ATG GCA ACC TAC TTT TCT
		Reverse	GCG **CTC GAG** TTA TTT CAC TTC TTT AAA T
13	HIAG00431	Forward	CCG **CCA TGG** GCC AAA ATG CTA AAC GT
		Reverse	CGC **CTC GAG** TTT TAA GTT TGC AAA AGC CT

No.	Gene No.	Pimer	Primer sequence (5'-3')
14	HIAG00789	Forward	CCC **CCA TGG** TGC GTT GTT TAG CAC T
		Reverse	CCG **CTC GAG** GCC ATA AAT TGT TCC T
15	HIAG01315	Forward	CGC **CCA TGG** TGT CAT TAC GCA TTA AAC
		Reverse	CCG **CTC GAG** GCC CAT ACG ATA GTT CGG T
16	HIAG01680	Forward	CCG **CCA TGG** TGC AAC AAC ACA ATC TCT
		Reverse	CCG **CTC GAG** AAT TCG CTC AAA ACC AGC T
17	HIAG01690	Forward	CCG **CCA TGG** TGC AAA AAC AGA TTG AAA T
		Reverse	CCC **CTC GAG** TTC TTC AAA ATA CCC CAT AT
18	HIAG00294	Forward	GCG **CCA TGG** TGA ATC AAA ATC TAA TTG
		Reverse	CGC **CTC GAG** TTC AAA CAA TTC CTT CAT
19	HIAG00956	Forward	GCG **CCA TGG** TGA AAC TTA CAT CGA AAG
		Reverse	GAG **CTC GAG** TTG ATT AAC TAA TAA AT
20	HIAG01692	Forward	GCG **CCA TGG** TGA AAA AAA CAC TTG CAG
		Reverse	CGC **CTC GAG** GTA AAC GCG TAA ACC TAC
21	HIAG01363	Forward	CGC **CCA TGG** AAA CGT ATT CAT TAT TAC
		Reverse	CGC **CTC GAG** CTC ACA TTG AAT TAT TAC

Table S2. Th17 Cell Antigens Selected by Bioinformatic Filters.

Protein	NT127 Locus	Rd KW20 gene ID	Known or inferred function	Size (aa)	Homology range for Hi strains[f]
0973[a, b]	HIAG_00973	HI0362	iron-chelated ABC transporter periplasmic-binding protein YfeA	293	96-99
1692[a]	HIAG_01692	HI0139	OmpP2, outer membrane protein P2	371	81-100
0956[b]	HIAG_00956	HI0379	iron-sulfur cluster assembly transcriptional regulator IscR	150	96-99
1363[b, c]	HIAG_01363	HI1249	ABC transporter periplasmic component, zinc utilization protein ZevA (1)	206	94-97
1677[b]	HIAG_01677	HI0119	Zinc ABC transporter, periplasmic-binding protein ZnuA	347	88-99
0758[b]	HIAG_00758	HI0408	Zinc ABC transporter, ATP-binding protein ZnuC	268	95-98
0789[b]	HIAG_00789	HI0144	NanK, N-acetylemannosamine kinase	300	97-99
1690[b]	HIAG_01690	HI0138	RnhA, ribonuclease HI	174	94-98
1680[b]	HIAG_01680	HI0122	MetC, cystathionine beta-lyase	395	92-99
0431[b]	HIAG_00431	HI0086	MetB, O-succinylhomoserine (thiol)-lyase	393	95-98
1315[b]	HIAG_01315	HI0221	GuaB, inosine-5'-monophosphate dehydrogenase	488	91-99
1342[b]	HIAG_01342	HI1277	Mrp, ATP-binding protein, chromosome partitioning	386	94-96
0293[b, d]	HIAG_00293	HI1087	YrbF, ABC transporter permease, membrane stability	261	95-99
0176[d, e]	HIAG_00176	HI0916	outer membrane protein H family member Omp26	197	93-99
0630[d]	HIAG_00630	HI1591	outer membrane lipoprotein carrier protein LolA	205	95-99
1097[c]	HIAG_01097	HI1300	ABC transporter ATP-binding protein uup	213	92-98
1360[c]	HIAG_01360	HI1252	ABC transporter ATP-binding protein	556	93-87
0259[a, c]	HIAG_00259	HI1124	oligopeptide permease ABC transporter membrane protein OppA	541	98-99
0264[c]	HIAG_00264	HI1119	membrane protein LapB*	292	97-100
1276[d]	HIAG_01276	HI0262	Heme-hemopexin utilization protein, HxuC	712	94-95

[a] *homologous to NTHi 86-028NP proteins identified in OMVs (2)*

[b] *required in NTHi/IAV coinfection (3)*

[c] *membrane protein (4)*

[d] *outer membrane protein (4, 5)*

[e] *homologous to OMP26 identified in (NTHI) strain 289 (6)*

[f] *the blast includes all the 15 completely sequenced Hi strains (Accession: CP002277.1, FQ670204.1, CP007471.1, CP000671.1, CP007472.1, CP000057.2, CP007470.1, CP007805.1, CP000672.1, L42023.1, CP005967.1, CP002276.1, FQ670178.1, CP008740.1, CP009610.1.)*

* *E. coli protein named LapB has been functionally characterized has no amino acid sequence similarity to LapB of NTHi*

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