Supplementary Methods

Bacterial strain

We used the kanamycin-resistant pneumococcal strain *S. pneumoniae* Xen10 (Caliper Life Sciences, Waltham, MA) derived from the wild type strain A66.1, which expresses PspA of family 1, clades 1 and 2.[1] The virulence of *S. pneumoniae* Xen10 is comparable with that of the parent strain.[2,3] The *S. pneumoniae* strain 3JYP2670, which express PspA of family 2, clade 4, was also used.[4] The *S. pneumoniae* strains were grown in brain heart infusion (BHI) broth at 37 °C in 5% CO_2.

MiRNA microarray analysis

The pre-immunized and post-booster serum samples were used for microarray analysis. Microarray analyses were performed by using the 3D-Gene miRNA microarray platform (TORAY, Kamakura, Japan).[5,6] RNA extraction was performed according to the manufacturer's instructions. Total RNA was labeled with Hy5 and hybridized at 32°C for 16 h on the 3D-Gene human miRNA chip. The oligonucleotide sequences of the probes were confirmed to the miRBase (http://www.mirbase.org).

We analyzed total of 2042 mature human miRNAs, which have the same sequences, or highly sequence homology with 544 miRNAs out of *Macaca mulatta* and 70 miRNAs out of *Macaca nemestrina*. MiRNA gene expression data were scaled by global normalization as described previously,[5,6] and eight miRNAs of which the levels were up-regulated in post-booster serum samples compared with pre-immunized serum samples were selected for further detailed analysis.

Supplementary References

1 Darrieux, M. *et al.* Fusion proteins containing family 1 and family 2 PspA fragments elicit protection against *Streptococcus pneumoniae* that correlates with antibody-mediated enhancement of complement deposition. *Infect. Immun.* 75, 5930-5938 (2007).

2 Francis, K. P. *et al.* Visualizing pneumococcal infections in the lungs of live mice using bioluminescent *Streptococcus pneumoniae* transformed with a novel gram-positive lux transposon. *Infect. Immun.* 69, 3350-3358 (2001).

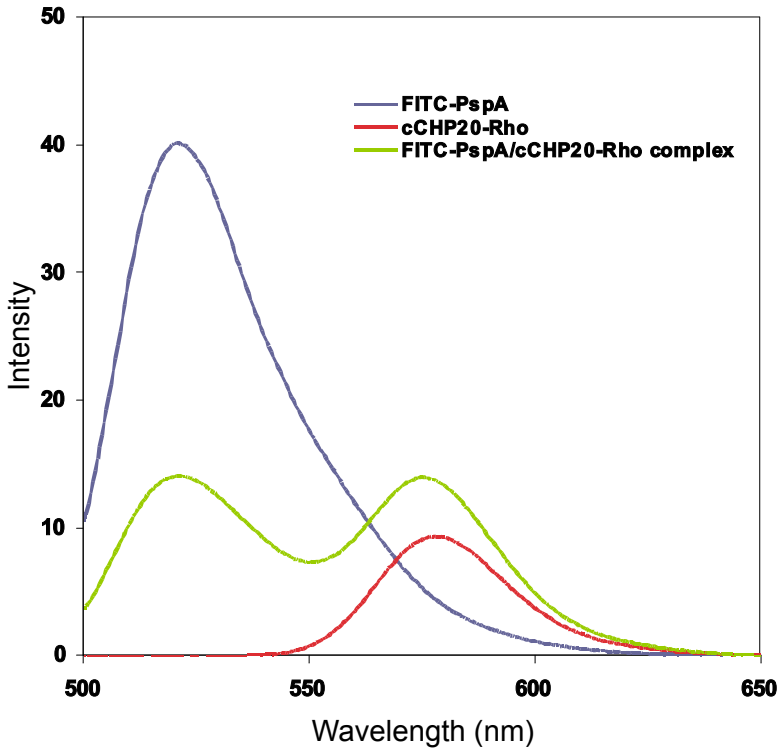
3 Kadurugamuwa, J. L. *et al.* Reduction of astrogliosis by early treatment of pneumococcal meningitis measured by simultaneous imaging, in vivo, of the pathogen and host response. *Infect. Immun.* 73, 7836-7843 (2005).

4 Briles, D. E. *et al.* Immunization of humans with recombinant pneumococcal surface protein A (rPspA) elicits antibodies that passively protect mice from fatal infection with Streptococcus pneumoniae bearing heterologous PspA. *J. Infect. Dis.* 182, 1694-1701 (2000).

5 Hisaoka, M. *et al.* Identification of altered MicroRNA expression patterns in synovial sarcoma. *Genes Chromosomes Cancer* 50, 137-145 (2011).

6 Nagino, K. *et al.* Ultrasensitive DNA chip: gene expression profile analysis without RNA amplification. *J. Biochem.* 139, 697-703 (2006).

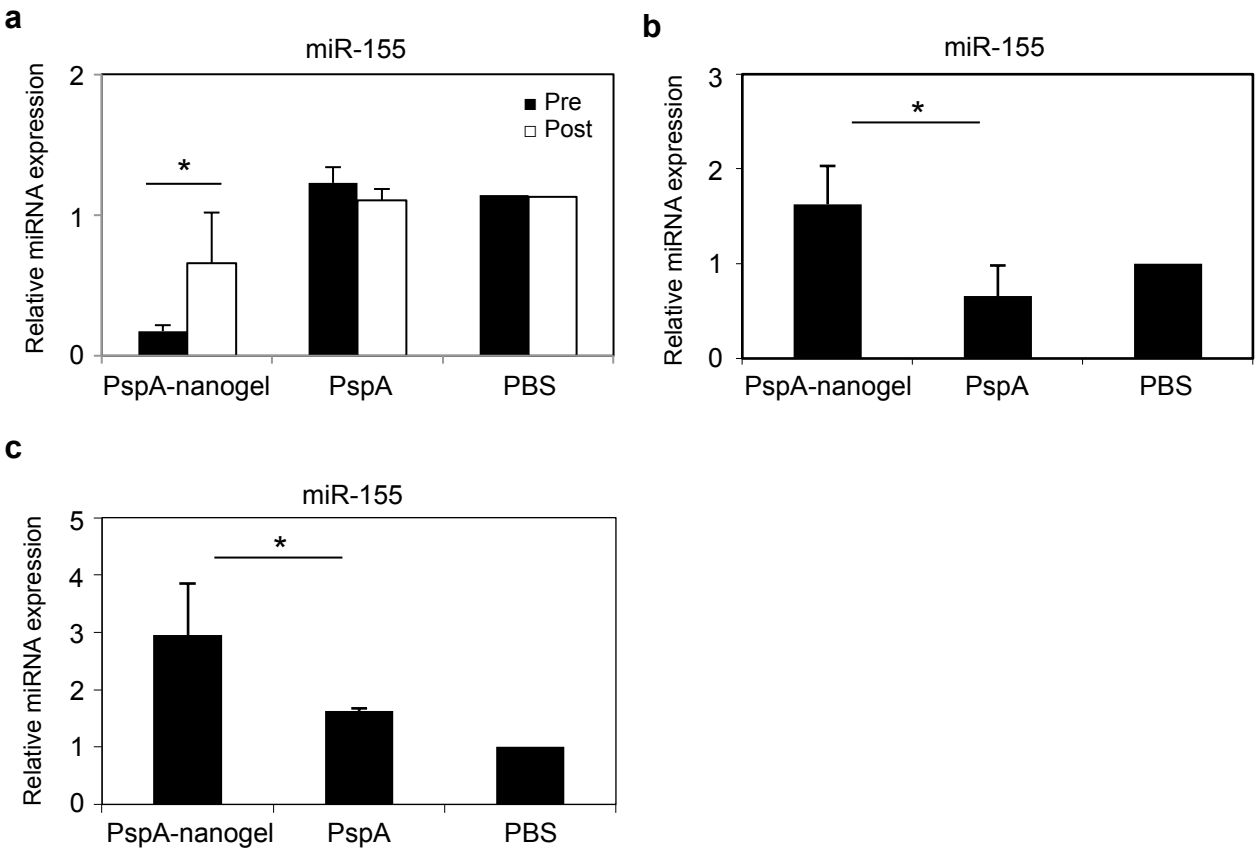
a



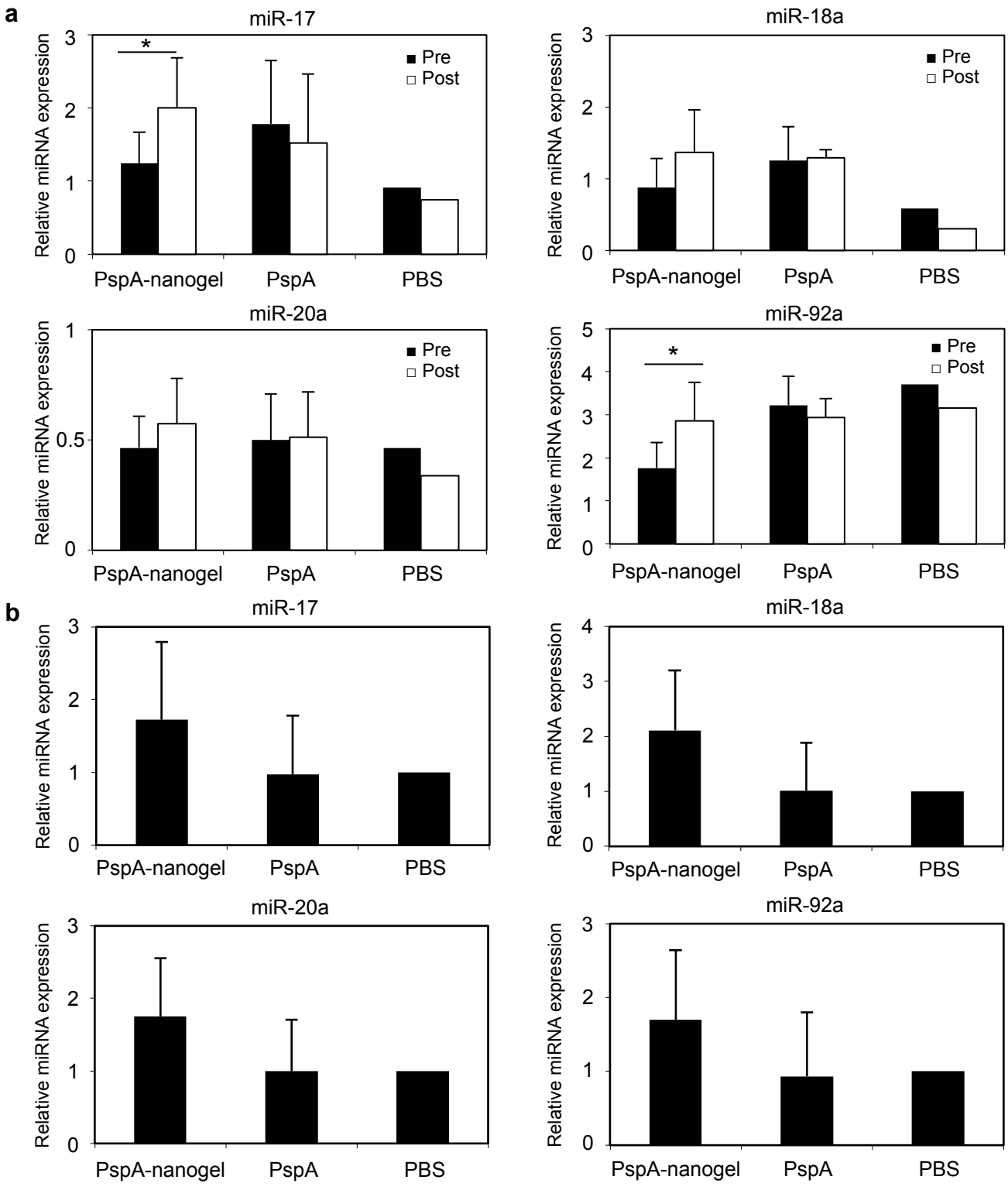
b

	D_H* (PDI**)	Zeta-potential
cCHP20	31.1 nm (0.179)	+2.8 mV
PspA/cCHP20	31.1 nm (0.176)	+1.3 mV

Supplementary Figure S1. The cCHP nanogel formed nanosize particles carrying vaccine antigen. (a) Fluorescence responses energy transfer (FRET) was detected from rhodamine-conjugated cCHP carrying FITC-conjugated PspA, but not FITC-conjugated naked PspA or rhodamine-conjugated naked cCHP. (b) Dynamic light scattering analysis showed that the cCHP nanogel particles were still of uniform size after the incorporation of PspA. The zeta-potentials of cCHP and PspA-cCHP were measured with a Zetasizer Nano ZS instrument (Malvern Instruments Malvern, UK). *: Hydrodynamic diameter, **: Polydispersity index.



Supplementary Figure S2. MiR-155 expression levels in sera (a), nasal tissues (b), and lung tissues (c) from nasally immunized macaques. The expression levels of miR-155 were analyzed by quantitative RT-PCR and normalized to the levels of miR-16. Values are shown as means ± SD in each experimental group. *$p < 0.05$, when compared between pre-immunization and post-booster groups (a), or PspA-nanogel and PspA/PBS groups in post-booster macaques (b and c).



Supplementary Figure S3. MiRNA expression levels in sera (a) and nasal tissues (b) from nasally immunized macaques. Expression levels of the indicated miRNAs were analyzed by quantitative RT-PCR and normalized to the levels of miR-16. Values are shown as the means ± SD in each experimental group. *$p < 0.05$, when compared between pre-immunization and post-booster groups.