Supporting Information

lymph node-targeted STING agonist nanovaccine against chronic HBV infection

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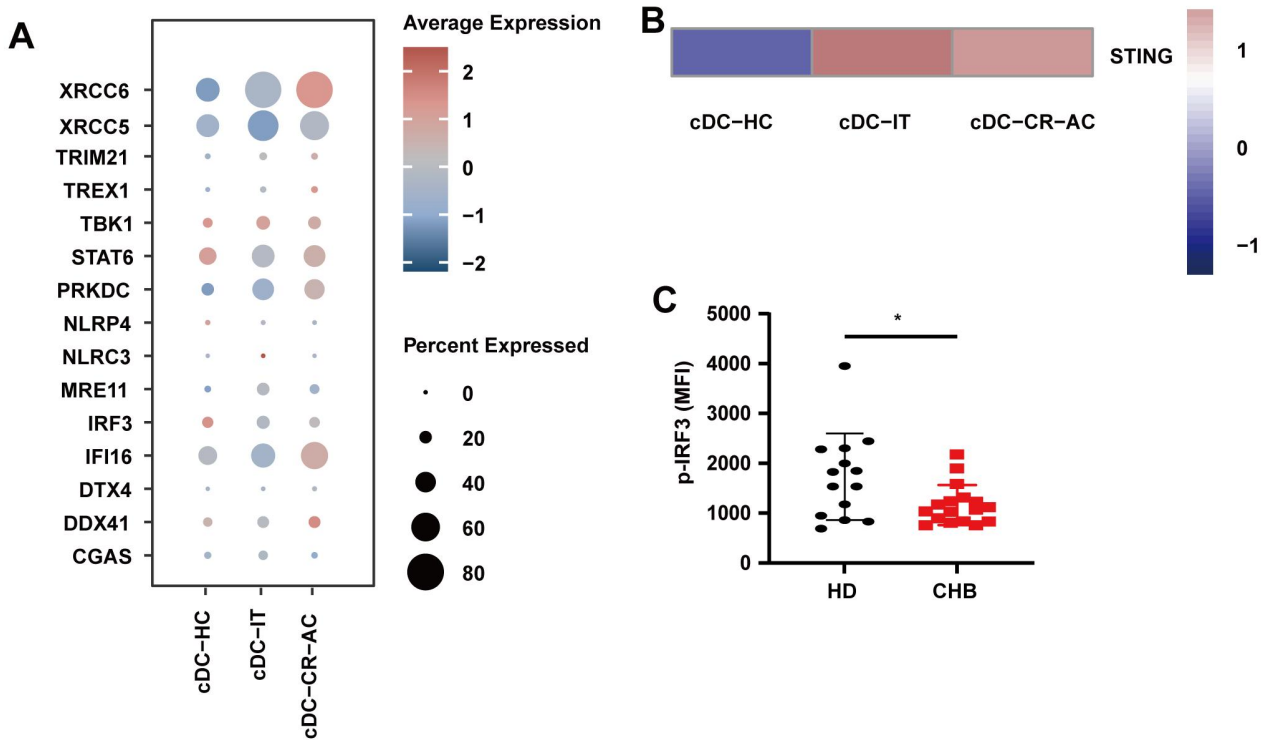
Running title: A STING nanovaccine against chronic HBV infection

Supplementary Table 1. Demographic and baseline characteristics of patients with immune-tolerant chronic hepatitis B and healthy donors

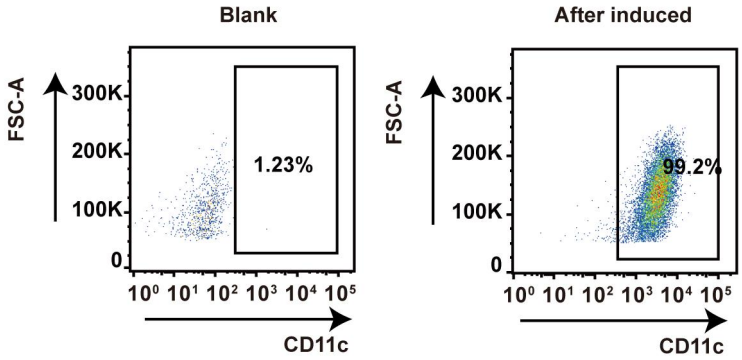
Variables	CHB patients (n=17)	Healthy donors (n=14)
Age, year	35.72 ± 1.55	43.23 ± 2.67
Gender, male(%)	8 (47.06%)	7 (50%)
ALT, IU/ml	23.55 (17.00, 45.00)	26.48 (15.00, 38.00)
AST, IU/ml	35.69 (29.00, 42.00)	26.54 (18.00, 38.00)
HBV DNA, $\log_{10}$ IU/ml	5.80 ± 0.17	Negative
HBsAg, $\log_{10}$ IU/ml	4.88 ± 0.15	Negative
HBeAg, $\log_{10}$ IU/ml	2.95 ± 0.34	Negative

Normally distributed data are presented as the mean ± SEM, and nonnormally distributed data are presented as the median (lower quartile, upper quartile). ALT, alanine transaminase; AST, aspartate aminotransferase; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen.

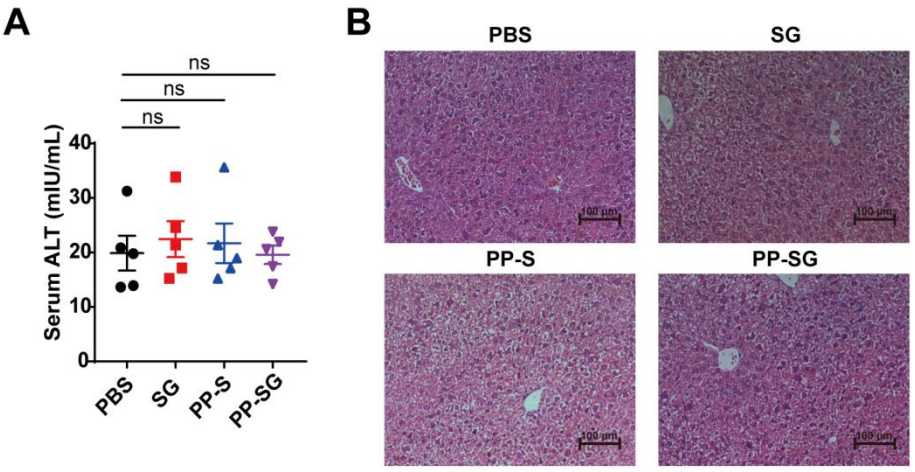
Supplementary Figure:



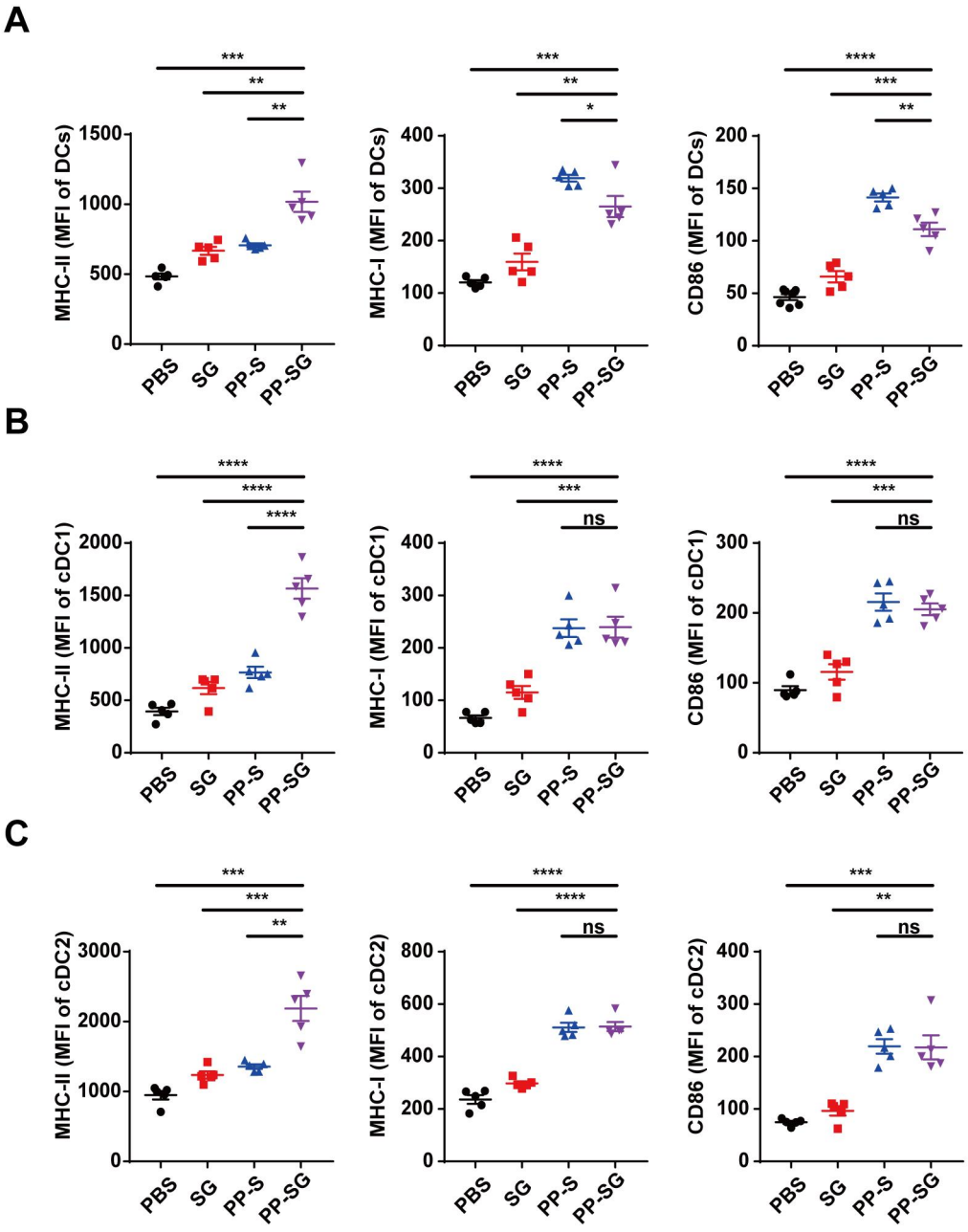
Supplementary Figure 1. CHB infection impaired the STING-mediated induction of host immune responses signal pathway compared with HD group. (A) Gene expression and (B) GSVA enrichment analysis associated with STING-mediated induction of host immune responses from GEO database (GSE182159). HC, healthy control; IT, immune tolerant (CHB); CR-AC, chronic resolved. (C) PBMCs were harvested from CHB patients and healthy donors (HDs) for flow cytometric analysis of p-IRF3 on $CD11c^{+}$ DCs. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.



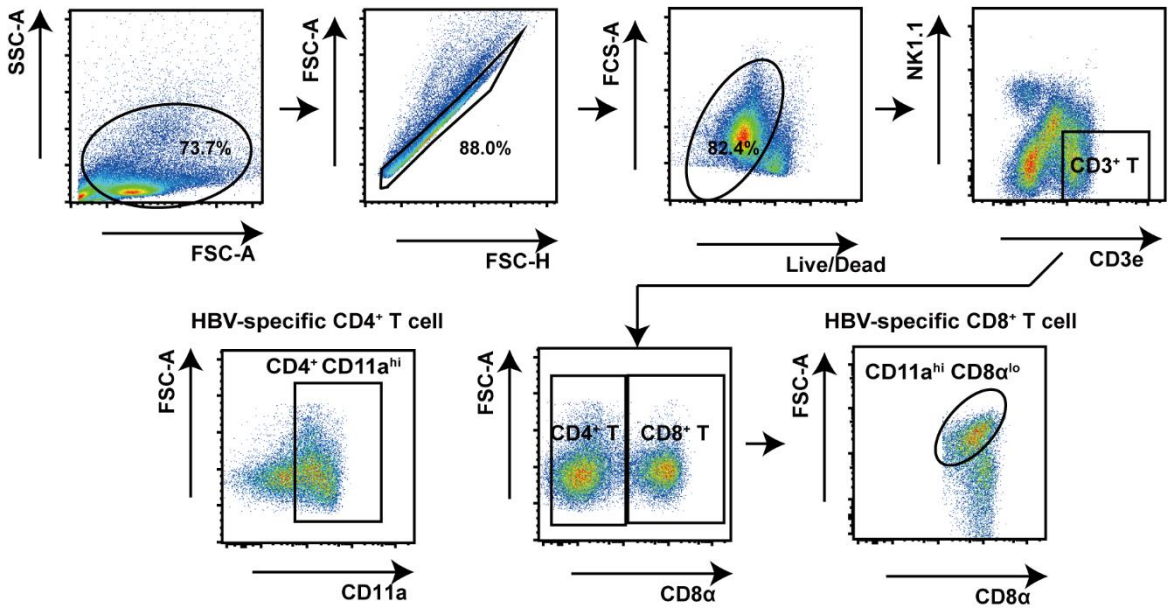
Supplementary Figure 2. The generation of BMDC *in vitro*. Murine bone marrow cells were stimulated with 10 ng/mL rmGM-CSF and 5 ng/mL rmIL-4 for 7 days *in vitro*. The percentage of $CD11c^+$ cells were analyzed by flow cytometry on day 7.



Supplementary Figure 3. PP-SG nanovaccine safely eliminated HBV in HBV-carrier mice. (A) Serum levels of ALT monitored on day 28 post-immunization. Serum ALT concentration below 40 mIU/mL was indicated physiologically normal. All data are expressed as mean ± SEM from biological replicates. (B) H&E staining was analyzed at day 28 post-immunization. SG is a physical mixture of HBsAg and c-di-GMP; PP-S is a nanovaccine containing HBsAg; PP-SG is a nanovaccine containing HBsAg and c-di-GMP. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.



Supplementary Figure 4. PP-SG nanovaccine activated splenic DCs in HBV-carrier mice. HBV-carrier mice treated with PBS or vaccines were re-challenged by 8 μg pAAV/HBV 1.2 on day 59. The following analyses were performed on day 7 after the HBV re-challenge. The expression of MHC-II, MHC-I, and CD86 on splenic DCs (A), cDC1s (B), and cDC2s (C). SG is a physical mixture of HBsAg and c-di-GMP; PP-S is a nanovaccine containing HBsAg; PP-SG is a nanovaccine containing HBsAg and c-di-GMP. The data are presented as a mean ± SEM (n ≧ 5). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.



Supplementary Figure 5. The gate strategy for CD8$^+$ T cell and CD4$^+$ T cells. The strategy to gating CD8$^+$ T cells and CD4$^+$ T cells from mononuclear cells in liver.