# Supplementary Materials

## An efficient antiviral strategy for targeting hepatitis B virus genome using Transcription Activator-Like Effector Nucleases

Jieliang Chen[1, 2], Wen Zhang[1, 2], Junyu Lin[1], Fan Wang[1, 2], Min Wu[2], Cuncun Chen[1, 3], Ye Zheng[2], Xiuhua Peng[2], Jianhua Li[1], and Zhenghong Yuan[1, 2, 3]

1. Key Laboratory of Medical Molecular Virology, School of Basic Medical Sciences, Shanghai Medical College of Fudan University, Shanghai 200032, China.
2. Shanghai Public Health Clinical Center affiliated to Fudan University, Shanghai 201508, China.
3. Institutes of Medical Microbiology and Biomedical Sciences, Fudan University, Shanghai 200032, China.

**Correspondence**
Prof. Zhenghong Yuan, M.D., Ph.D.
E-mail: zhyuan@shaphc.org
Add: Room 1115, Zhidao Building, 138 YiXueYuan Road, Shanghai 200032, China
Tel: +86-21-54237669
Fax: +86-21-64227201

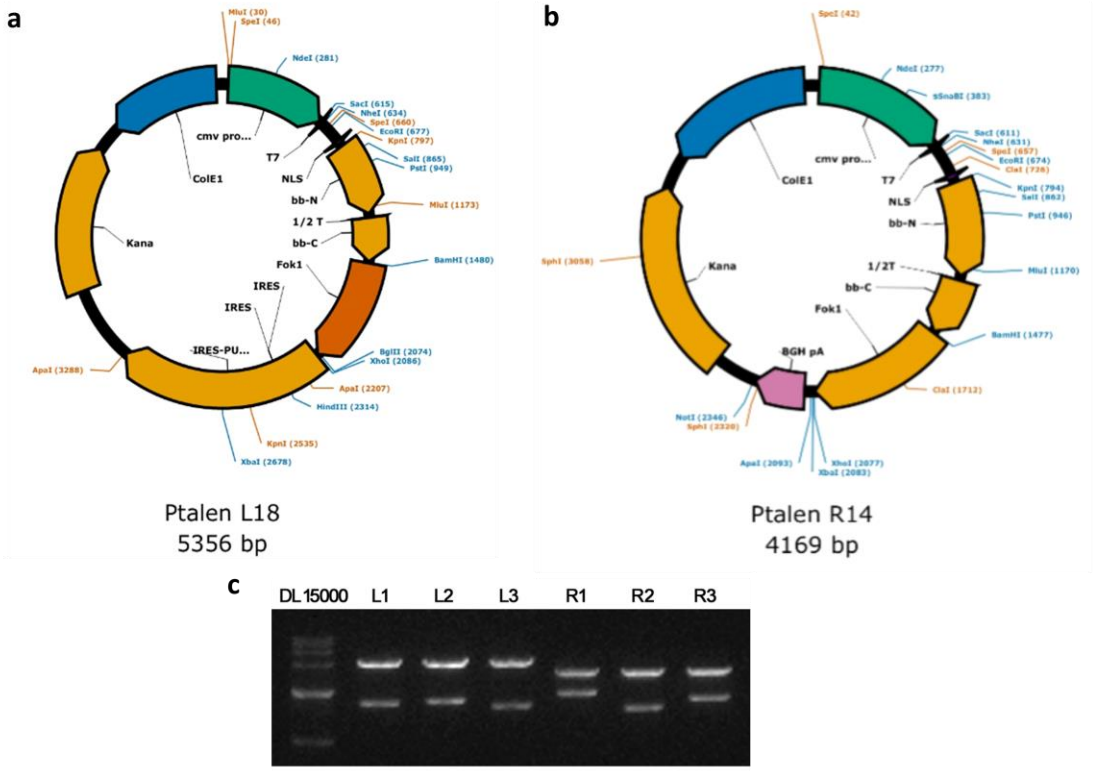


**Fig S1. Construction of plasmids carrying the coding sequence of designed TALENs.**

**(a, b)** The structure of the plasmid used to express the L (left) (a) and the R (right) (b) TALENs (provided by SIDANSAI Biotechnology). **(c)** The constructed plasmids were double-digested with the restriction endonucleases PstI and BamHI and subjected to 1% agarose-gel electrophoresis to identify the inserted TALENs sequences.

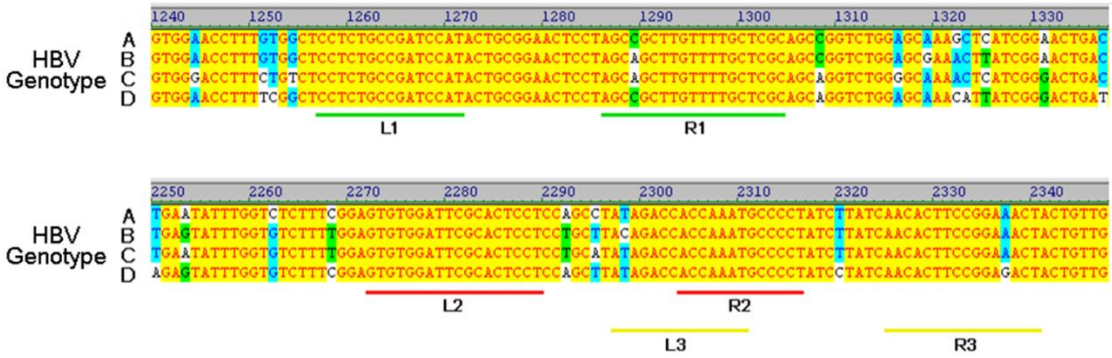


**Fig S2. Designed TALENs for conserved regions of viral DNA among different genotypes.**

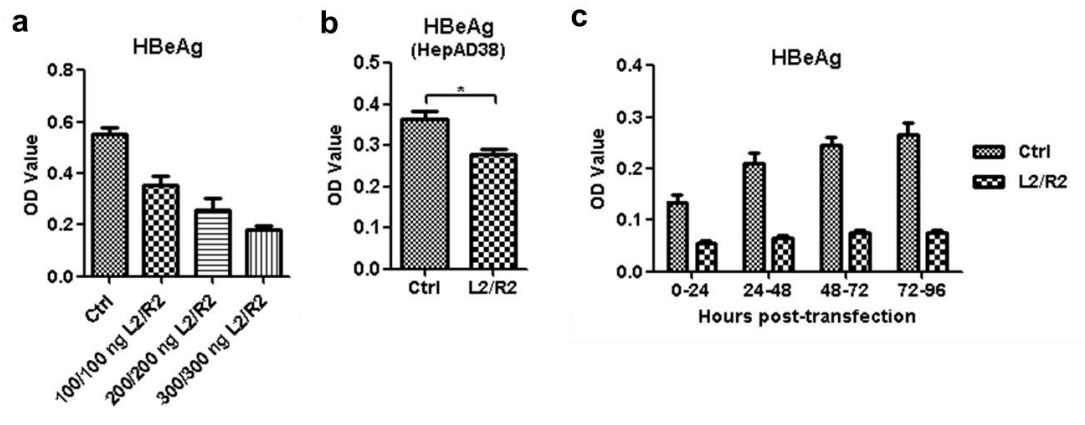


**Fig S3. Suppression of HBeAg production by TALENs.**

**(a)** Huh7 cells in 24-well plates were co-transfected with the linear full-length HBV DNA and indicated amounts of the plasmids encoding TALENs-L2 and -R2. 48 h post transfection, the supernatants were collected to detect HBeAg by ELISA.

**(b)** HepAD38 cells were firstly cultured with tetracycline (1 μg/ml) for 1 week. The cells were then plated into 24-well plates and the tetracycline was added back to cultures. The control plasmids or plasmids encoding TALENs-L2/R2 and pEGFPs were transfected into the cells at day 1, 3 and 5 post plating using GeneTran (Biomiga) and the medium were refreshed every 2 days. The culture supernatants of the cells at day 7 were collected to detect HBeAg by ELISA. The transfection efficiency among the two groups was approximately 40% at day 7 as determined by the observation of GFP fluorescence.

**(c)** Huh7 cells in 24-well plates were co-transfected with the linear full-length HBV DNA and plasmids encoding TALENs-L2 (200 ng) and -R2 (200 ng). The supernatants of the cells were collected to detect HBeAg by ELISA at indicated times.

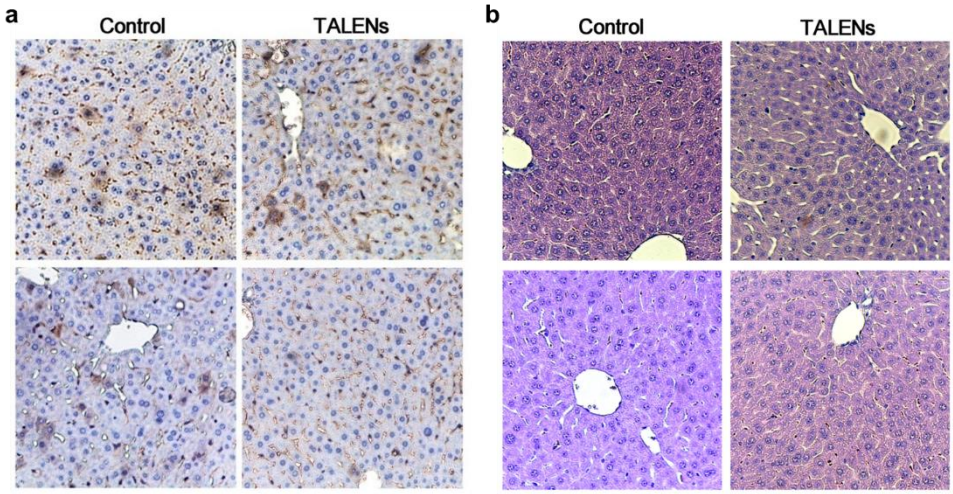


**Fig S4. HBcAg expression and immunohistochemical analysis in the mice liver sections.**

**(a)** Immunohistochemical staining for HBcAg in liver sections of the mice from control and TALENs group. **(b)** HE staining of the liver sections of the mice hydrodynamically-injected with the linear full-length HBV DNA and the control vector or the plasmids encoding TALENs-L2 and -R2 at day 6 post-injection.

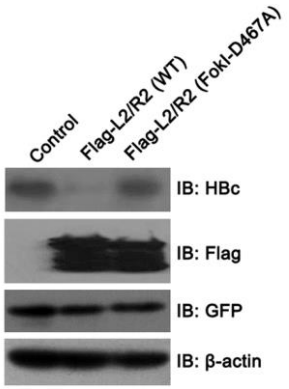


**Fig S5. The inhibitory effect of TALENs is largely dependent on the FokI endonuclease activity.**

Plasmids encoding Flag-TALE-FokI with single alanine substitution mutations at Asp-467 of FokI (Single amino acid substitutions uncouple the DNA binding and strand scission activities of Fok I endonuclease. Proc Natl Acad Sci U S A. 1993 Oct 15;90(20):9596-600.) were constructed and identified by nucleotide sequencing. Huh7 cells were co-transfected with the linear full-length HBV DNA and the indicated plasmids. 48 h post transfection, the cells were lysed to examine the HBcAg and Flag-TALE-FokI expression.

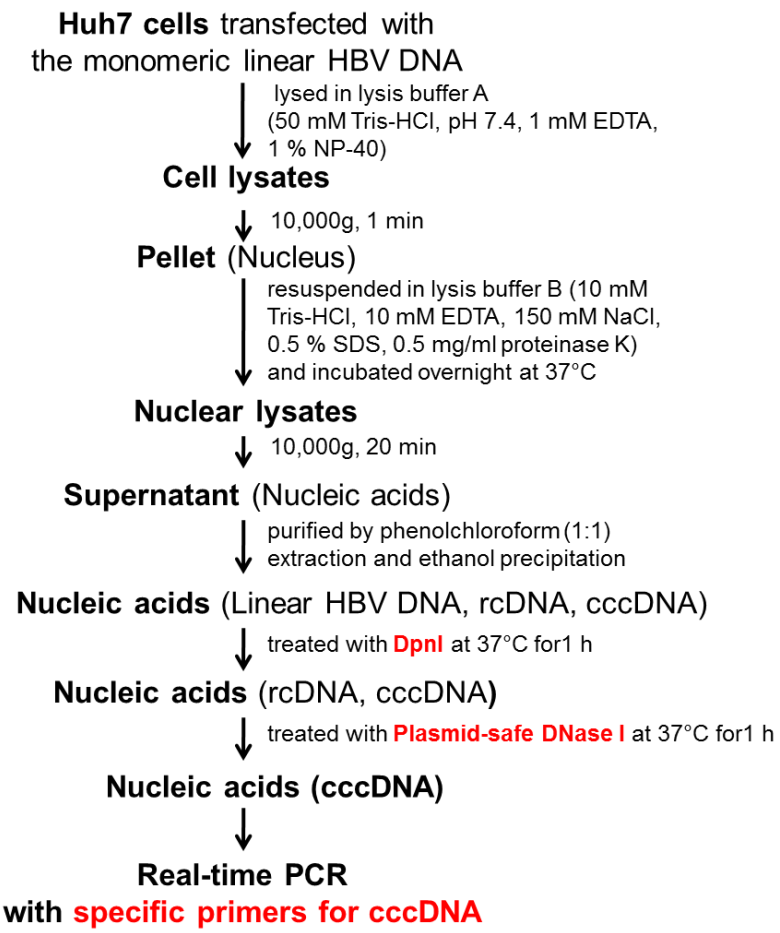


**Fig S6. The main steps of extraction and quantification of the intracellular HBV cccDNA.**