

Supplementary Information for

Extracellular vesicles secreted by HBV-infected cells modulate HBV persistent in hydrodynamic HBV transfection mouse model

Masatoshi Kakizaki^{1,2}, Yuichiro Yamamoto², Motoyuki Otsuka³, Kouichi Kitamura⁴, Masatoshi Ito⁵, Hideki Derek Kawai⁶, Masamichi Muramatsu⁴, Tatehiro Kagawa⁷ and Ai Kotani^{1,2*}.

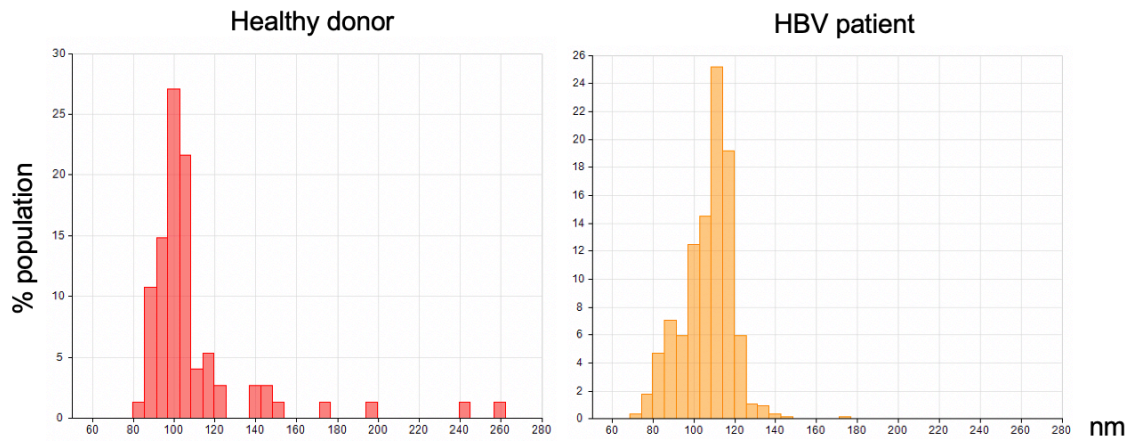
From the ¹Department of Innovative Medical Science, Tokai University School of Medicine, Kanagawa, 259–1193, Japan; ²Division of Hematological Malignancy, Institute of Medical Sciences, Tokai University, Kanagawa, 259–1193, Japan; ³Department of Gastroenterology, Graduate school of Medicine, The University of Tokyo, Tokyo, 113-8655, Japan; ⁴Department of Virology II, National Institute of Infectious Diseases, Tokyo, 162-8640, Japan; ⁵Support Center for Medical Research and Education, Tokai University School of Medicine, Kanagawa, 259-1193, Japan; ⁶Department of Science and Engineering for Sustainable Innovation, Faculty of Science and Engineering, Soka University, Hachioji, Tokyo, 192-8577, Japan; ⁷Division of Gastroenterology and Hepatology, Department of Internal Medicine, Tokai University School of Medicine, Kanagawa, 259-1193, Japan

*To whom correspondence should be addressed: Ai Kotani, Department of Hematological Malignancy, The Institute of Medical Sciences, Tokai University, Department of Innovative Medical Science, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa, 259-1193, Japan; aikotani@k-lab.jp; Tel. 81.463-93-1121 ext. 2781,

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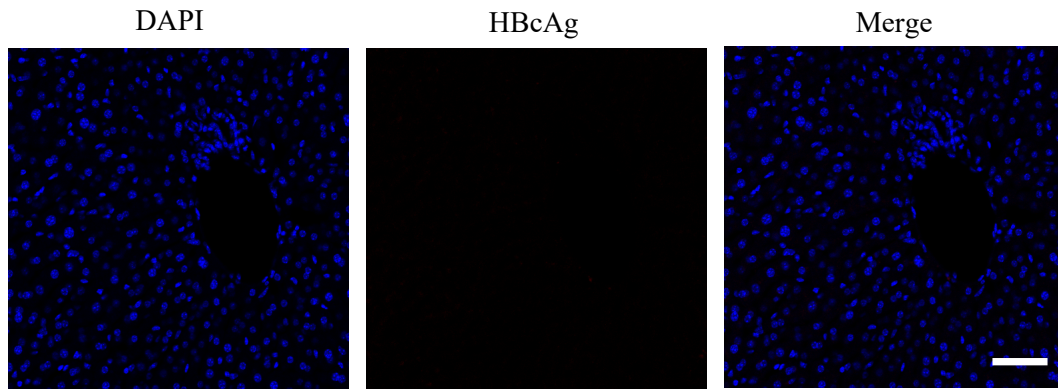
Figures S1 to S6

Fig S1 Characterization of EVs isolated from healthy donor and HBV patient



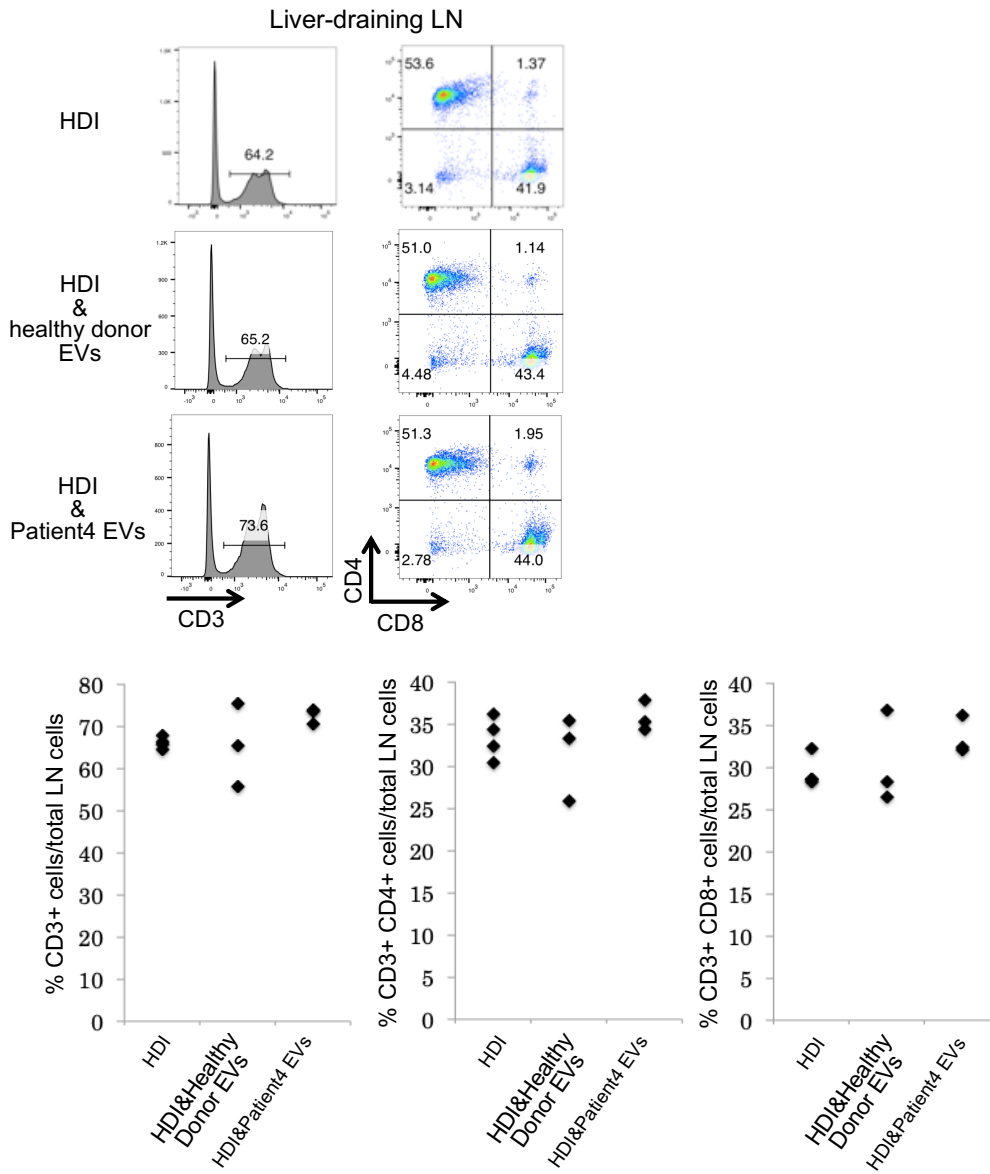
qNano analysis of EVs isolated from healthy donor and HBV patient.

Fig S2. HBcAg expression in the liver of mouse treated with only patient EVs.



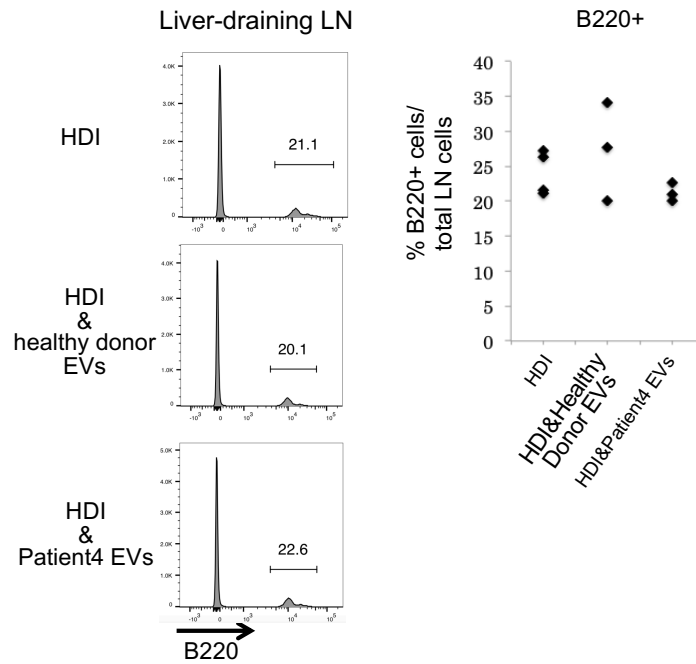
Immunofluorescence of mouse liver sections obtained 3 days after injection of only patient 4 EVs. Sections were stained to detect HBcAg (Red) and nuclei using DAPI (Blue). White bar indicate 50 μm .

Fig S3. Ratio of CD3+ T cells in liver-draining LN and spleen after injection of EVs from healthy donor and HBV patient.



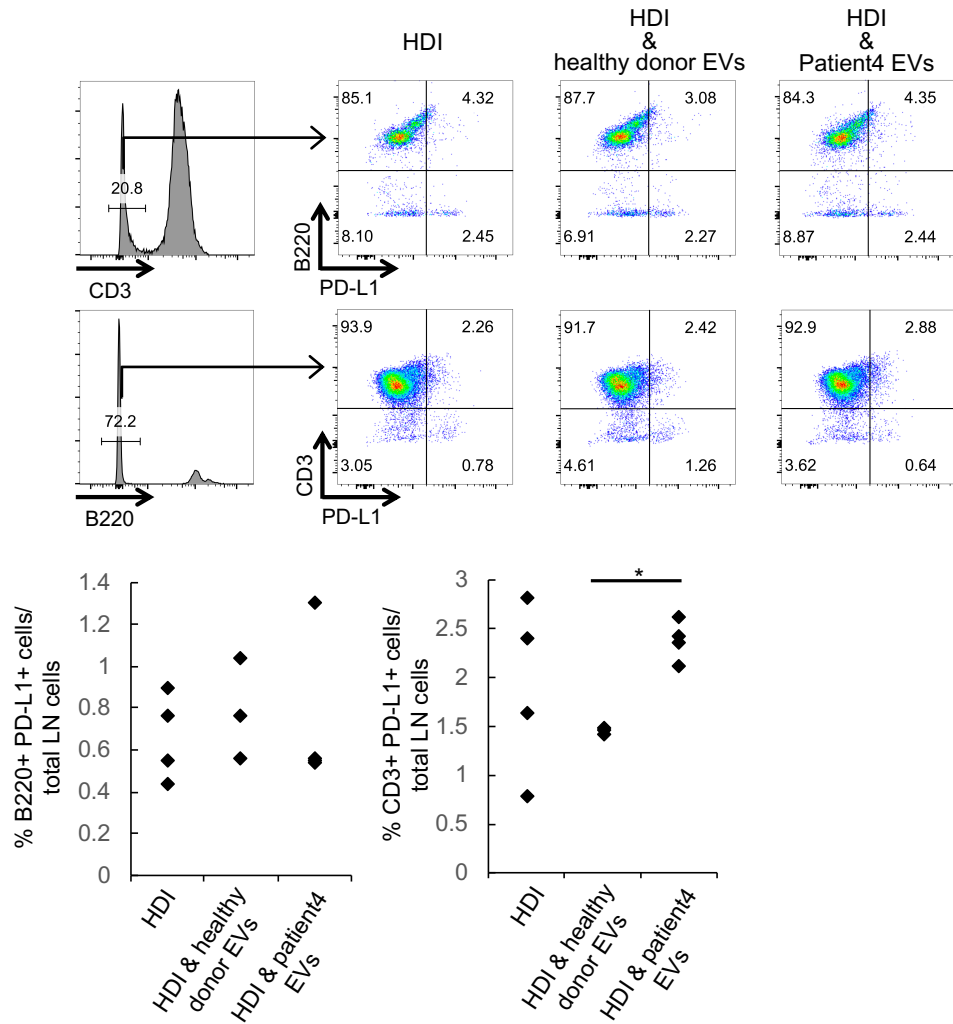
Cells from liver-draining LN and spleen were isolated 3 days after HDI and analyzed. The frequencies of CD3+ CD4+ T cells and CD3+ CD8+ T cells in mice treated with HDI alone, HDI and healthy donors EVs, and HDI and HBV patients EVs were detected by flow cytometry (top) and plotted in a graphic (bottom).

Fig S4. Ratio of B220+ B cells in liver-draining LN after injection of EVs from healthy donor and HBV patient.



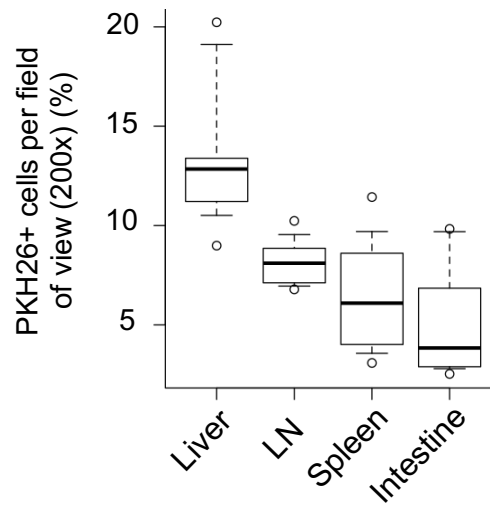
Cells from liver-draining LN were isolated 3 days after HDI and analyzed. The frequencies of B220+ B cells in mice treated with HDI alone, HDI and healthy donors EVs, and HDI and HBV patients EVs were detected by flow cytometry (left) and plotted in a graphic (right).

Fig S5. PD-L1 expression in CD3+ T cells and B220+ B cells of liver-draining LN after injection of EVs from HBV patient.



Cells from liver-draining LN were isolated 3 days after HDI and analyzed. The frequencies of B220+ PD-L1+ cells and CD3+ PD-L1+ cells in mice treated with HDI alone, HDI and healthy donors EVs, and HDI and HBV patients EVs were detected by flow cytometry (top) and plotted in a graphic (bottom).

Fig S6. Systemic biodistribution of the EVs derived from HBV-infected cells.



PKH-26 labeled EVs secreted from HBV-infected cells were injected in the tail vein of mice, and after 24 hours, cryosections were prepared for liver, liver-draining LNs, spleen and intestine and observed with a confocal laser microscopy. PKH-26 positive cells were counted in 15 fields from 3 mice. Ratio to total cells were calculated.