# Increased Infiltration of Regulatory T Cells in Hepatocellular Carcinoma of Patients with Hepatitis B Virus Pre-S2 Mutant

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**Supplementary Figure Legends**

**Supplementary Figure S1. Detection and quantification of CTLs in HCC tissues of patients.** (A) CTLs in liver tissues of HBV-related HCC patients were detected by fluorescent IHC staining with antibodies against CD3 (Anti-CD3) and CD8 (Anti-CD8). CTLs that were double positive for CD3 (green in color) and CD8 (red in color) appeared yellow, as indicated by white arrows in the single-color and merged images. Nuclei were counterstained with DAPI (blue in color). Shown were representative results. Original magnification, ×40. Scale bar, 200 μm. (B) Whole-slide image of the liver tissue section stained by H&E to define the tumor region, as highlighted by the black circle. Shown was a representative image. Scale bar, 5 mm. (C) Magnification of the tumor region outlined by the white rectangle box in the top-right image of the H&E-stained liver tissue section. Original magnification, ×40. Scale bar, 100 μm. (D) Whole-slide image of the liver tissue section stained by fluorescent IHC with CD3 and CD8 antibodies. Tumor region was highlighted by the red circle. Shown was a representative merged image. Scale bar, 5 mm. (E) Magnification of the tumor region outlined by the white crossed lines in the top-right image and the white rectangle box in the down-right image of the fluorescent

IHC-stained liver tissue section. CTLs were double positive for CD3 (green in color) and CD8 (red in color) and appeared yellow, as indicated by white arrows. Nuclei were counterstained with DAPI (blue in color). Shown was a representative merged image. Original magnification, ×40. Scale bar, 200 μm.

**Supplementary Figure S2. Detection and quantification of granzyme B-expressing cells in HCC tissues of patients.** (A) Granzyme B in liver tissues of HBV-related HCC patients was detected by fluorescent IHC staining with granzyme B antibody (Anti-granzyme B). Cells that were positive for granzyme B (green in color) were indicated by white arrows in the single-color and merged images. Nuclei were counterstained with DAPI (blue in color). Shown were representative results. Original magnification, ×40. Scale bar, 200 μm. (B) Whole-slide image of the liver tissue section stained by H&E to define the tumor region, as highlighted by the black circle. Shown was a representative image. Scale bar, 5 mm. (C) Magnification of the tumor region outlined by the white rectangle box in the top-right image of the H&E-stained liver tissue section. Original magnification, ×40. Scale bar, 100 μm. (D) Whole-slide image of the liver tissue section stained by fluorescent IHC with

granzyme B antibody. Tumor region was highlighted by the red circle. Shown was a representative merged image. Scale bar, 5 mm. (E) Magnification of the tumor region outlined by the white rectangle box in the right image of the fluorescent IHC-stained liver tissue section. Granzyme B-expressing cells appeared green as indicated by white arrows. Nuclei were counterstained with DAPI (blue in color). Shown was a representative merged image. Original magnification, ×40. Scale bar, 200 μm.

**Supplementary Figure S3. Detection and quantification of Foxp3-expressing cells in HCC tissues of patients.** (A) Foxp3 in liver tissues of HBV-related HCC patients was detected by fluorescent IHC staining with Foxp3 antibody (Anti-Foxp3). Cells that were positive for Foxp3 (green in color) were indicated by white arrows in the single-color and merged images. Counterstaining of Foxp3-positive nuclei (green in color) with DAPI (blue in color) appeared cyan. Shown were representative results. Original magnification, ×40. Scale bar, 200 μm. (B) Whole-slide image of the liver tissue section stained by H&E to define the tumor region, as highlighted by the black circle. Shown was a representative image. Scale bar, 6 mm. (C) Magnification of the tumor region outlined by the white rectangle box in the top-right image of the

H&E-stained liver tissue section. Original magnification, ×40. Scale bar, 100 μm. (D) Whole-slide image of the liver tissue section stained by fluorescent IHC with Foxp3 antibody. Tumor region was highlighted by the red circle. Shown was a representative merged image. Scale bar, 5 mm. (E) Magnification of the tumor region outlined by the white rectangle box in the right image of the fluorescent IHC-stained liver tissue section. Foxp3 (green in color)-expressing cells were counterstained with DAPI (blue in color) and appeared cyan in nuclei as indicated by white arrows. Shown was a representative merged image. Original magnification, ×40. Scale bar, 200 μm.