EBV-associated epithelial cancers cells promote vasculogenic mimicry formation via a secretory cross-talk with the immune microenvironment

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Supplementary figures

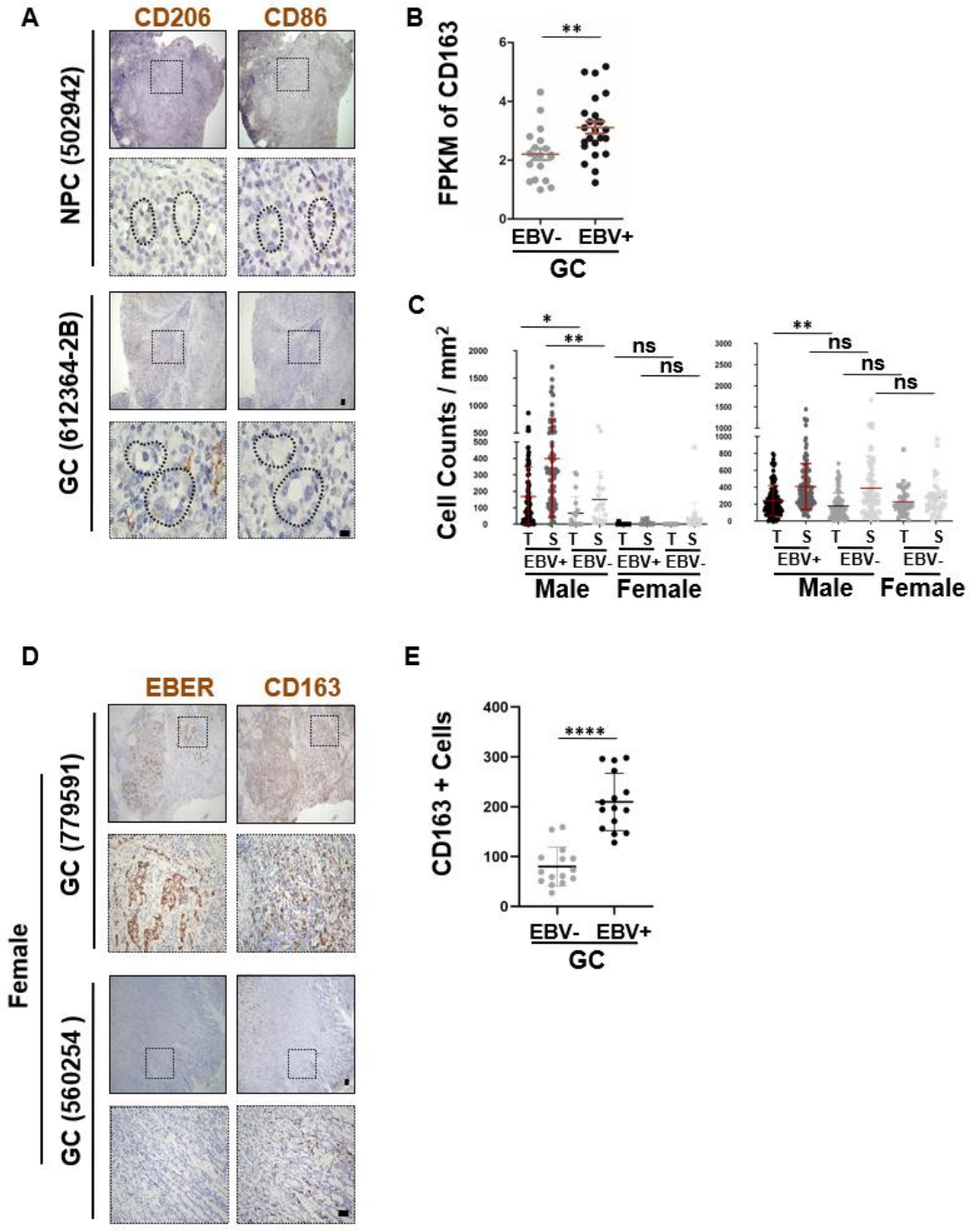

Fig. S1 The Markers of macrophages surrounded the VM. **A.** (top) NPC and (bottom) EBV-positive gastric carcinoma (GC) serial sections were stained with antibodies targeting human CD206 and CD86. Short

black scale bars = 100 μm, long black scale bars = 10 μm. **B.** The FPKM values of the CD163 in 19 EBV-negative and 24 EBV-positive gastric carcinoma cases by transcriptome sequencing. Mean ± SD, two-tailed unpaired t-test. **p < 0.01. **C.** IHC scores of the indicated macrophage cells in two EBV-negative and six EBV-positive male NPC biopsies (left) or in six EBV- negative and ten EBV- positive gastric carcinoma biopsies (right). Means ± SD, two-tailed Mann–Whitney test. ns: not significant, *p < 0.05, **p < 0.01. **D.** EBV-positive and -negative female gastric carcinoma (GC) serial sections were stained with antibodies targeting human EBER and CD163. Short black scale bars = 100 μm, long black scale bars = 10 μm. **E**. Number of CD163 positive cells counted as in D. Mean ± SD, n=5, two-tailed unpaired t-test, ****p < 0.0001.

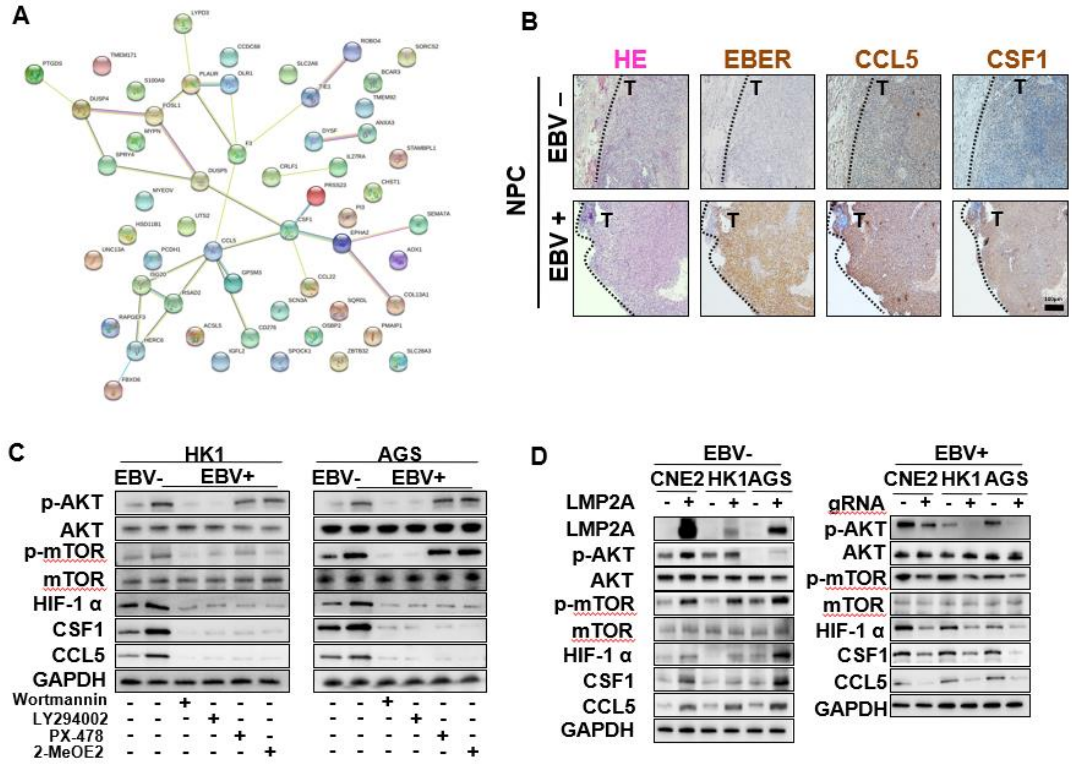

Fig. S2 EBV-infected epithelial cancer cells favour secretion of macrophage modulatory factors via the AKT/mTOR/HIF-1α pathway. **A.** Top 10 candidate core genes from the PPI network of the DEGs. **B.** Serial sections of EBV-negative and EBV-positive NPC tissues were stained with H&E, EBER and antibodies targeting CCL5 and CSF1. Scale bars = 500 μm. **C.** CNE2, HNE1, and AGS cells were transfected with empty vector or construct encoding LMP2A. Activation of the AKT/mTOR/HIF-1α signalling pathway and the levels of two macrophage modulatory factors (CSF1 and CCL5) were determined by immunoblotting. **D.** Immunoblots of the AKT/mTOR/HIF-1α signalling pathway and two macrophage modulatory factors (CSF1 and CCL5) of EBV- and EBV+ HK1 or AGS cells following LY294002 (50 μM), Wortmannin (1 μM), PX-478 (1 μM), and 2MeOE2 (1 μM) treatment for 12 h.

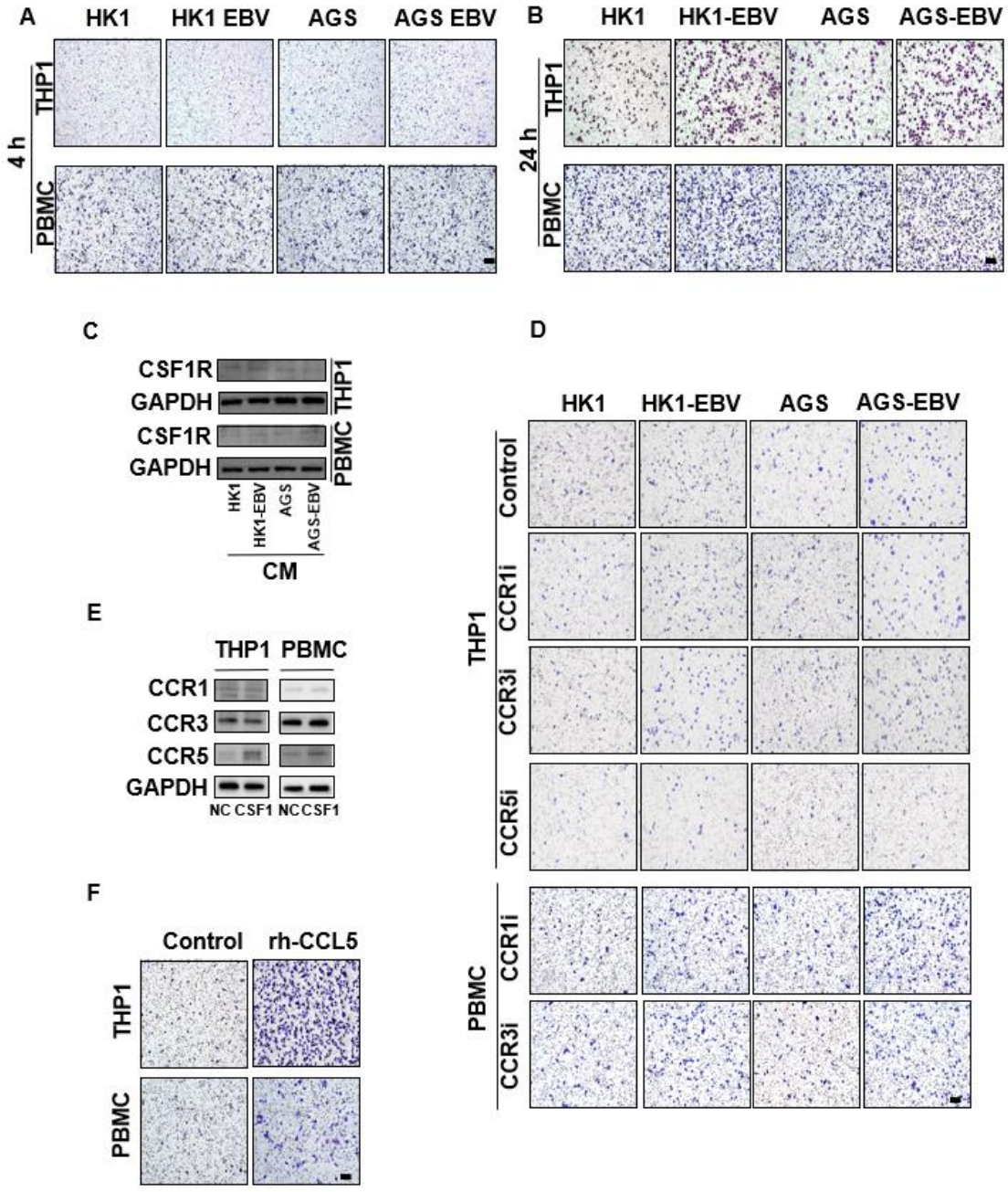

Fig. S3 EBV-associated epithelial cancer cells increase monocyte migration. A. Images of monocyte migration induced by CM of EBV-infected epithelial cancer cells and their parental cells. Images were taken 4 h after seeding on chamber. Scale bars = 100 μm. **B.** Images of monocyte

migration induced by CM of EBV-infected epithelial cancer cells and their parental cells. Images were taken 24 h after seeding on chamber. Scale bars = 100 μm. **C.** CSF1R levels on the THP1 and PBMC-derived monocyte stimulated by EBV-positive tumour cells CM after 24 h. **D.** Images of monocyte migration induced by CM of EBV-infected epithelial cancer cells and their parental cells in the presence of CCR1, CCR3, and CCR5 inhibitor and rh-CCL5. Images were taken 4 h after seeding on chamber. Scale bars = 100 μm. **E.** Levels of CCR1, CCR3, and CCR5 on the THP1 and PBMC-derived monocyte stimulated by CSF1 after 24 h. **F.** Images of monocyte migration induced by recombinant human CCL5 (rh-CCL5). Scale bars = 100 μm.

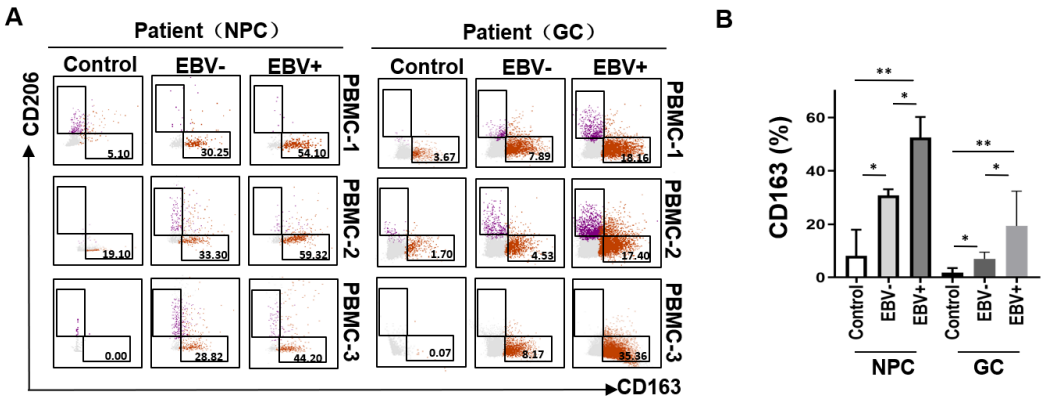

Fig. S4 EBV-associated epithelial cancer cells induce M2c-like macrophages differentiation. A. Flow cytometry dot plots from PBMC-derived monocytes of three donor showing CD163 and CD206 levels after treatment with CM of patient derived EBV-infected and EBV-uninfected epithelial cancer cells. **B.** %CD163 levels after treatment with CM of

patient derived EBV-infected and EBV-uninfected epithelial cancer cells. Mean ± SD, two-tailed unpaired t-test. *p < 0.05, **p < 0.01.

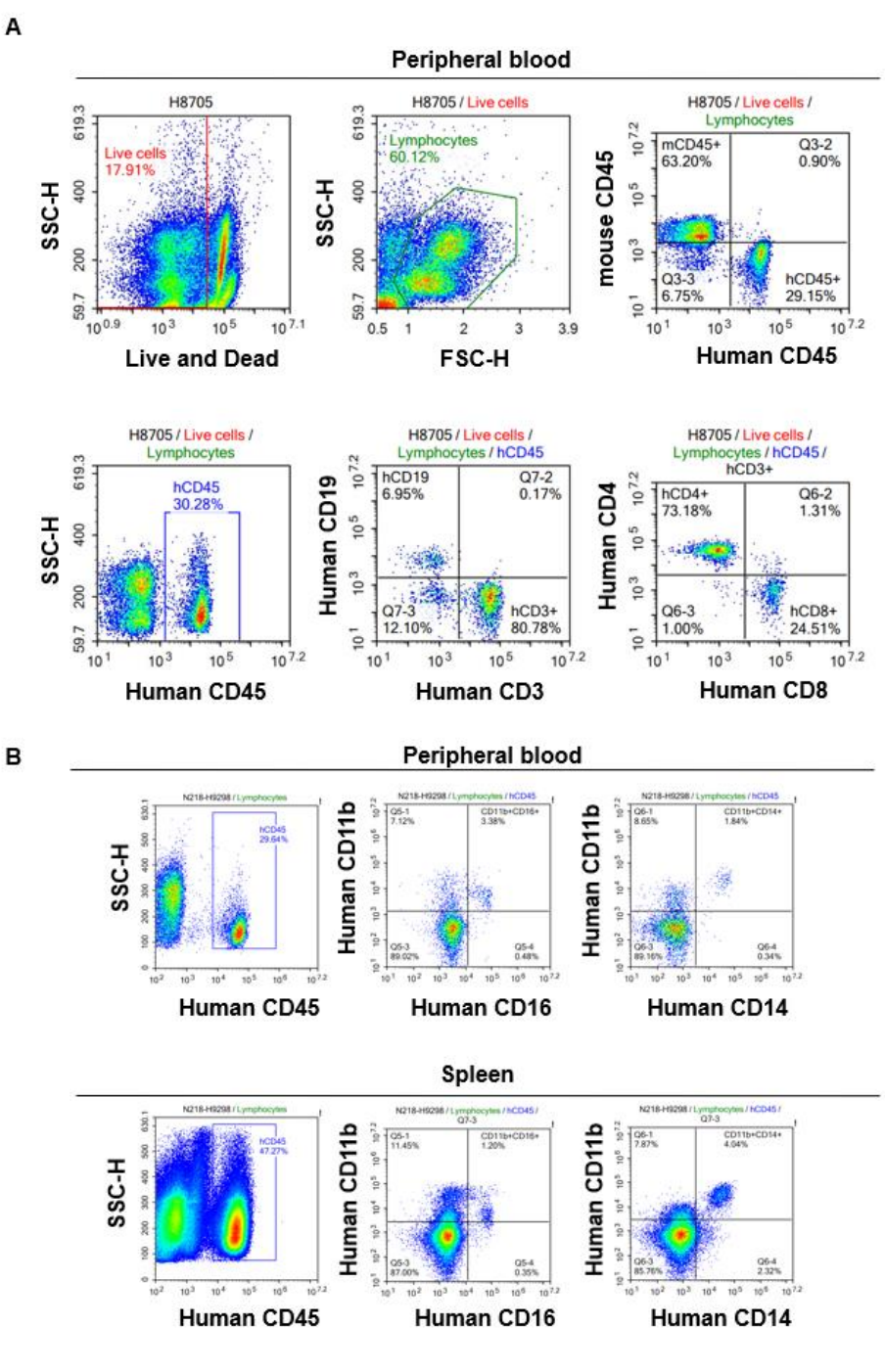

Fig. S5 Humanised HLA-A24 transgenic NSG mice. A. Flow cytometry dot plots from peripheral blood of one humanised HLA-A24 transgenic NSG mice showing CD45, CD3, CD19, CD4, and CD8 levels. **B.** Flow

cytometry dot plots from peripheral blood and spleen of one humanised HLA-A24 transgenic NSG mouse showing CD45, CD16, CD14, and CD11b levels.