Supporting Information for

Original article

VEGFR2-targeted antibody fused with IFN*α*mut regulates the tumor microenvironment of colorectal cancer and exhibits potent anti-tumor and anti-metastasis activity

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Supplement 1

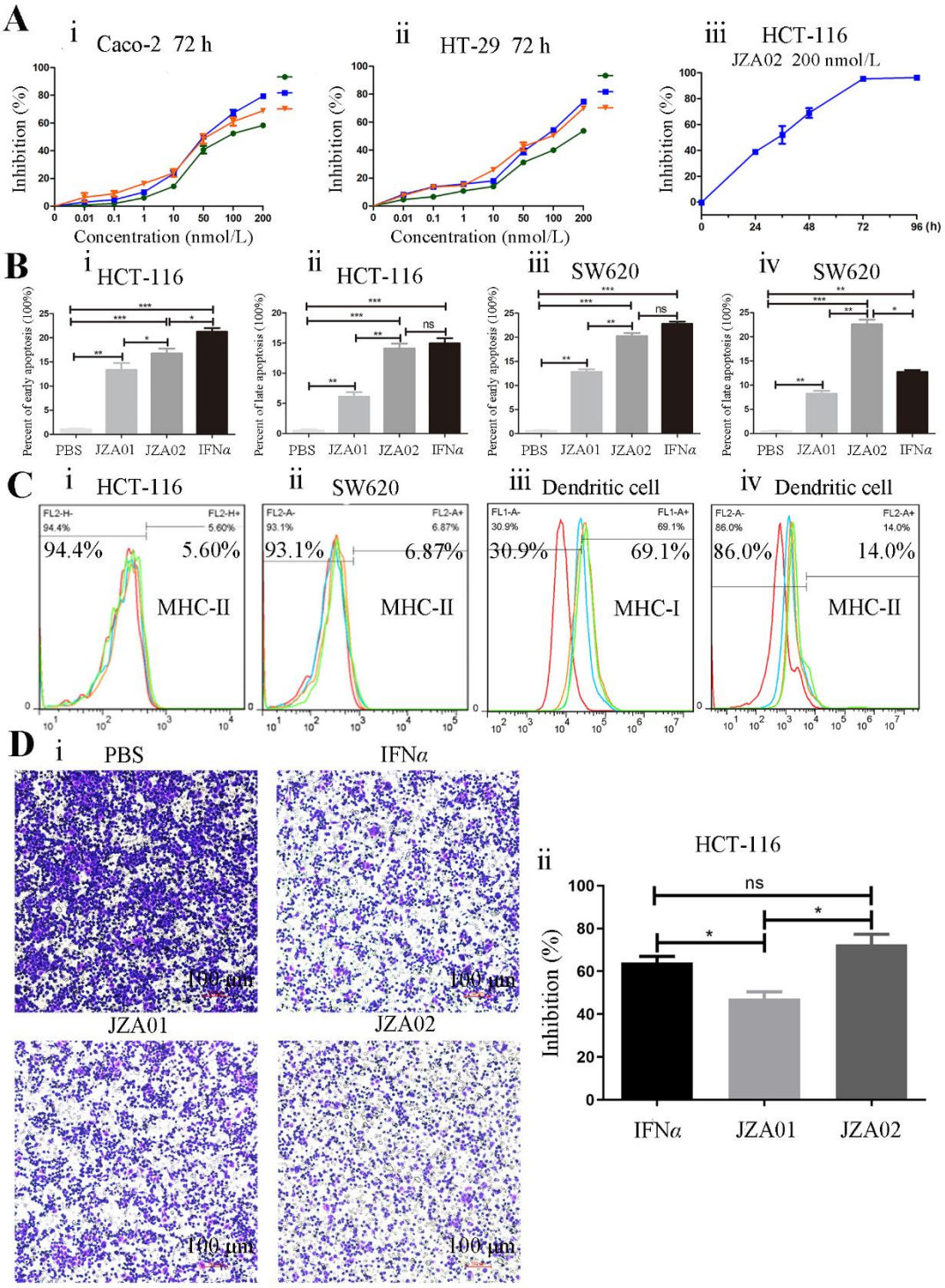

Figure S1 (A) (i) and (ii) HT-29 and CACO-2 cells were treated with IFN*α* (200 nmol/L), JZA01(100 nmol/L), or JZA02(100 nmol/L) for 72 h, and an MTT assay was used to detect the metabolic activity of the surviving cells. Inhibition of cell growth by IFN*α*, JZA01, and JZA02 was plotted with the growth of untreated controls set to 100%. (iii) HCT-116 cells were treated with JZA02 (200 nmol/L) for 24, 36, 48,

72, and 96 h, and an MTT assay was used to detect the metabolic activity of the surviving cells. Inhibition of cell growth by IFNα, JZA01, and JZA02 was plotted with the growth of untreated controls set to 100%. (B) The statistical analysis of apoptosis rate of each group with early apoptosis and late apoptosis in Fig. 2A. (C) Flow cytometry assay of the expression of MHC class II of HCT-116 and SW620 cells under different treatments (JZA01 100 nmol/L, JZA02 100 nmol/L, and IFNα 200 nmol/L) for 48 h. Immature dendritic cells were isolated from non-activated hPBMCs and incubated under different treatments for 72 h. The expression of MHC class I and MHC class II in dendritic cells was detected by flow cytometry assay. The red, green, orange, and blue lines represent PBS treatment, JZA02 treatment, IFNα treatment, and JZA01 treatment, respectively (D) (i) Photomicrographs and quantitative analysis of a cell migration assay indicated that JZA02 suppressed the migration of HCT-116 cells better than IFNα or JZA01. The count of invasive cells treated with different drugs was measured by Image-Pro Plus 6.0. Inhibition (%)=[(control–experiment)/control] ×100. (ii) The statistical and quantitative analysis of (i) results. Data are presented as the mean ± SD, n=3; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

Supplement 2

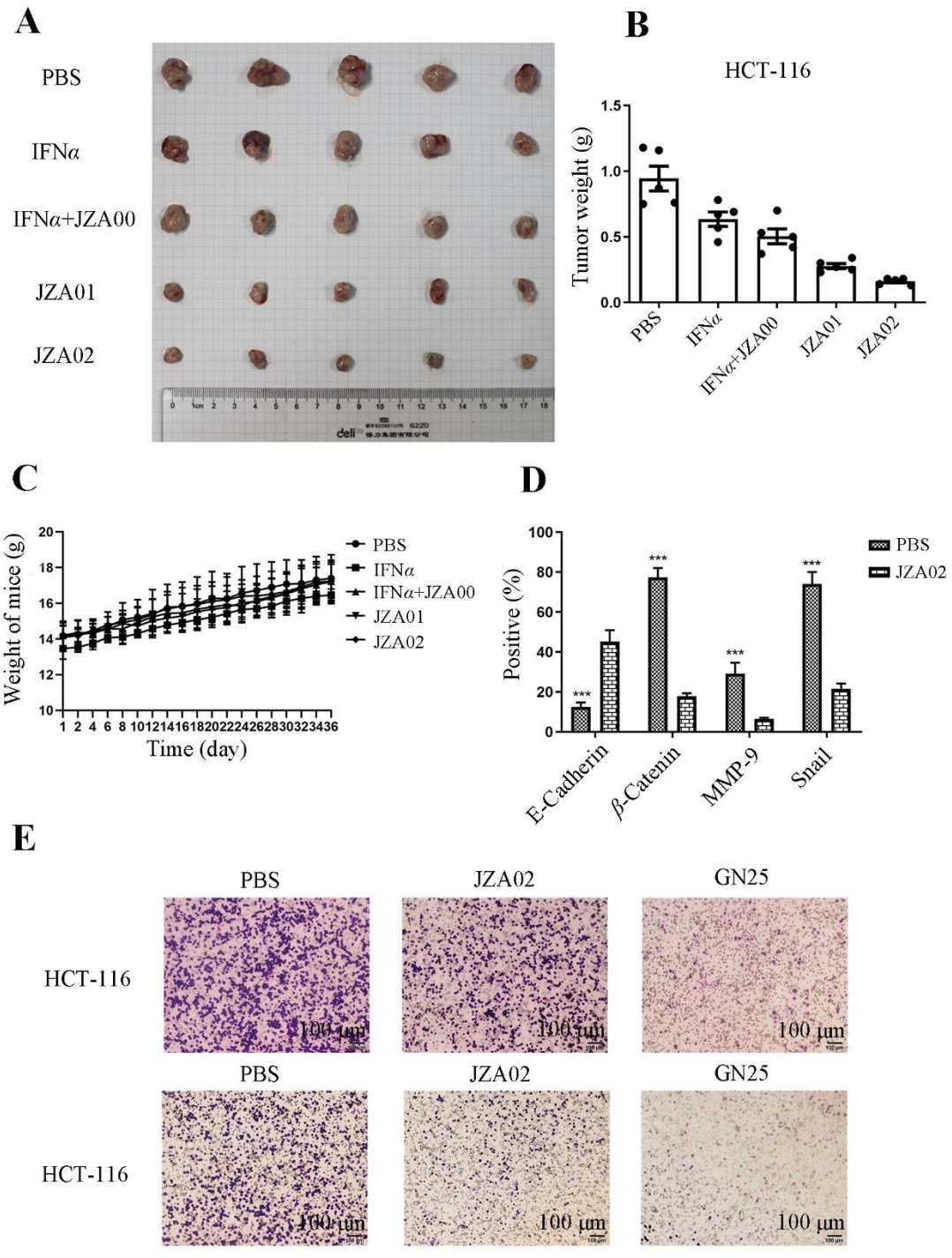

Figure S2 (A) and (B) Tumor tissues from every group were photographed and weighed. (C) The mouse weight was monitored from Day 1 to Day 36. (D) The statistical and quantitative analysis of Fig. 6A. (E) Photomicrographs of a cell migration assay and a transwell invasion assay indicated that GN25 (a novel inhibitor of snail–P53 binding) suppressed the migration and invasion of HCT-116 cells.