Figure S1. Representative dot plots used to quantify circulating tumor cells. The crucial procedure involved flow cytometric staining with EpCAM and its isotype, each obtained from 2 ml of whole blood. The gating strategy comprises the following steps: (A and E) Hoechst+ cells were gated to exclude cell fragments from all events. (B and F) Singlet cells were gated to mitigate false positive outcomes stemming from cell aggregation. (C and G) CD45+ cells were subsequently excluded to minimize residual white blood cell contamination. Prior to CTC enumeration, (D) EpCAM+ cells and (H) their corresponding isotype were separately gated. CTC, circulating tumor cells; FSC-H, forward scatter-height; FSC-A, forward scatter-area; APC, allophycocyanin; PE, phycoerythrin.

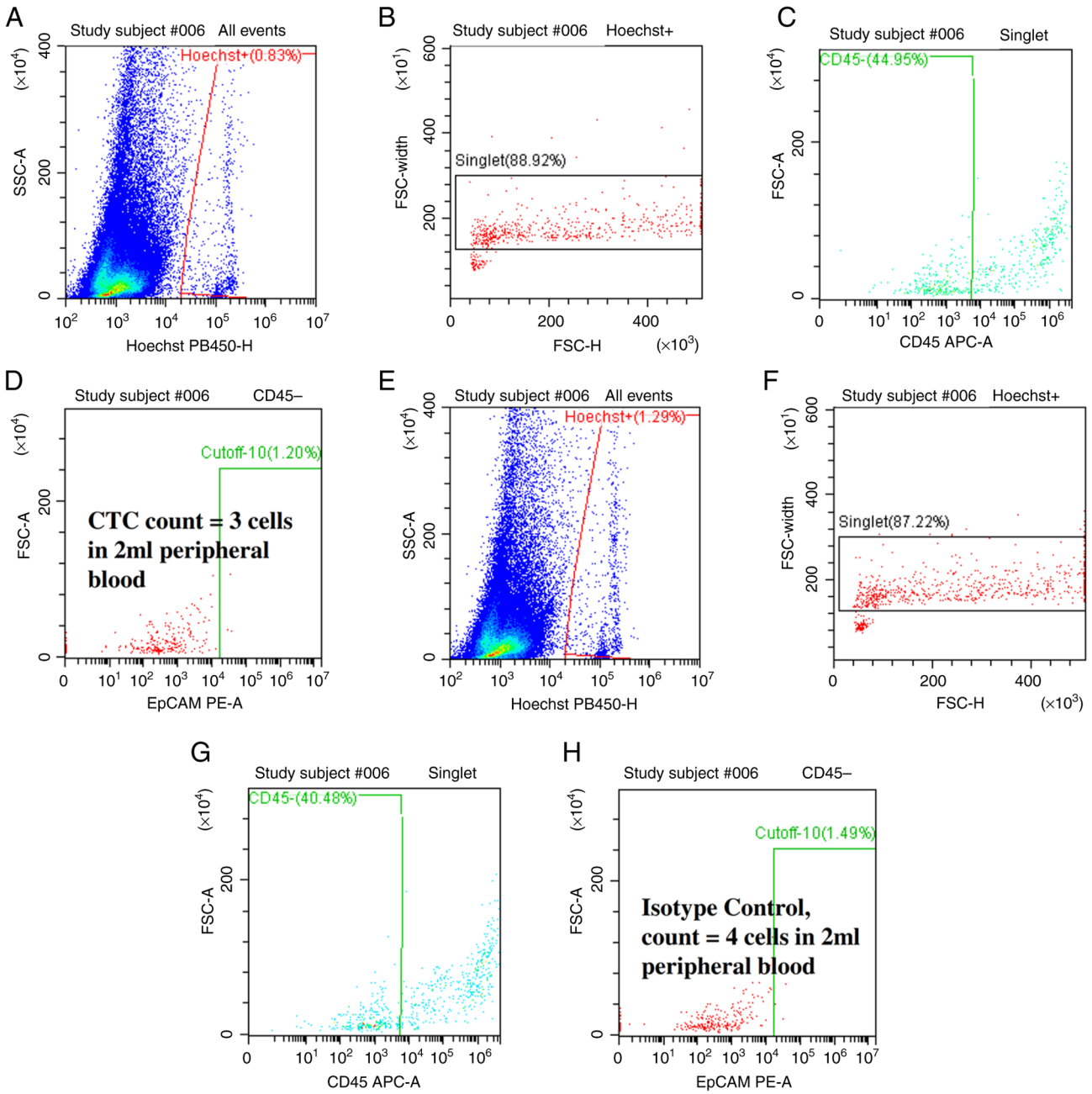

Figure S2. Demonstration of the presence of circulating tumor cells by immunofluorescence staining. (A) HeLa, (B) H1975 and (C) WBCs (scale bars, 50 μm), as well as (D) patient-CTCs (arrows; scale bars, 20 μm). CTC, circulating tumor cell; EpCAM, epithelial cell adhesion molecule; WBC, white blood cells.