Figure S1

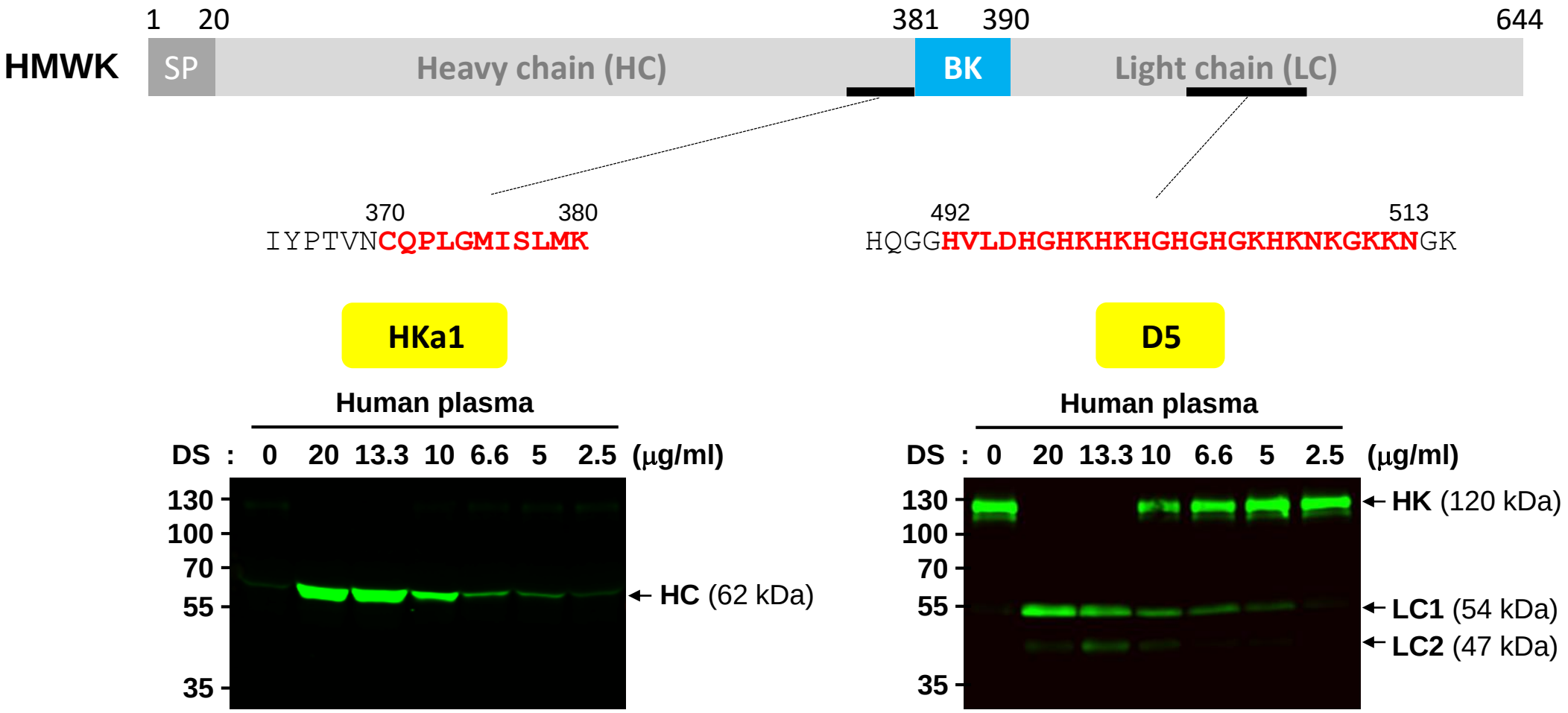

Figure S1. Affinity-purified polyclonal rabbit anti-human HK antibodies used in this study. Schematic diagram of human high molecular weight kininogen (HK) showing amino acid sequences (red) of peptides used to raise affinity-purified rabbit polyclonal antibodies. HKa1, heavy chain-specific antibody that specifically recognizes the C-terminus of the HK heavy chain (HK: ~62 kDa) exposed after cleavage of bradykinin from intact HK; D5, light chain-specific antibody that recognizes the free HK light chain released from cHK after reduction (two bands, LC1: ~54 kDa, LC2: ~47kDa) as well as the light chain within intact HK (~120 kDa).

Figure S2

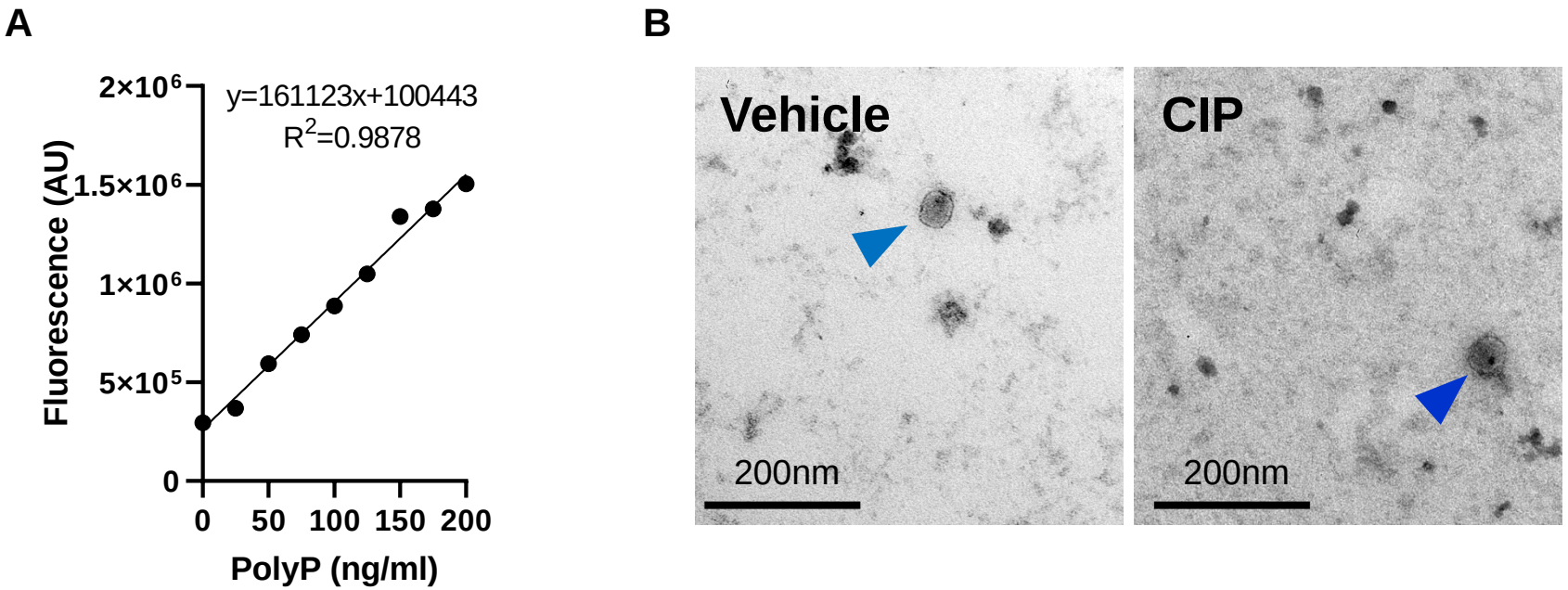

Figure S2. PolyP standard curve and electron microscopy of EV before and after CIP treatment. (A) Standard curve for polyP quantification developed using long-chain polyP as standard. (B) Representative EM image of L3.6 EV treated with CIP or buffer control.

Figure S3

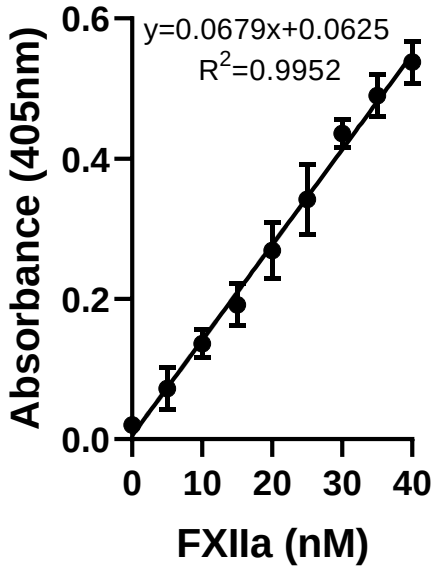

Figure S3. FXIIa standard curve. Various concentrations of FXIIa (0, 5, 10, 15, 20, 25, 30, 35, and 40 nM) was added to normal human plasma in the presence of S-2302 and incubated under identical conditions as used to assess FXII activation EV. A405 was measured to prepare a standard curve.

Figure S4

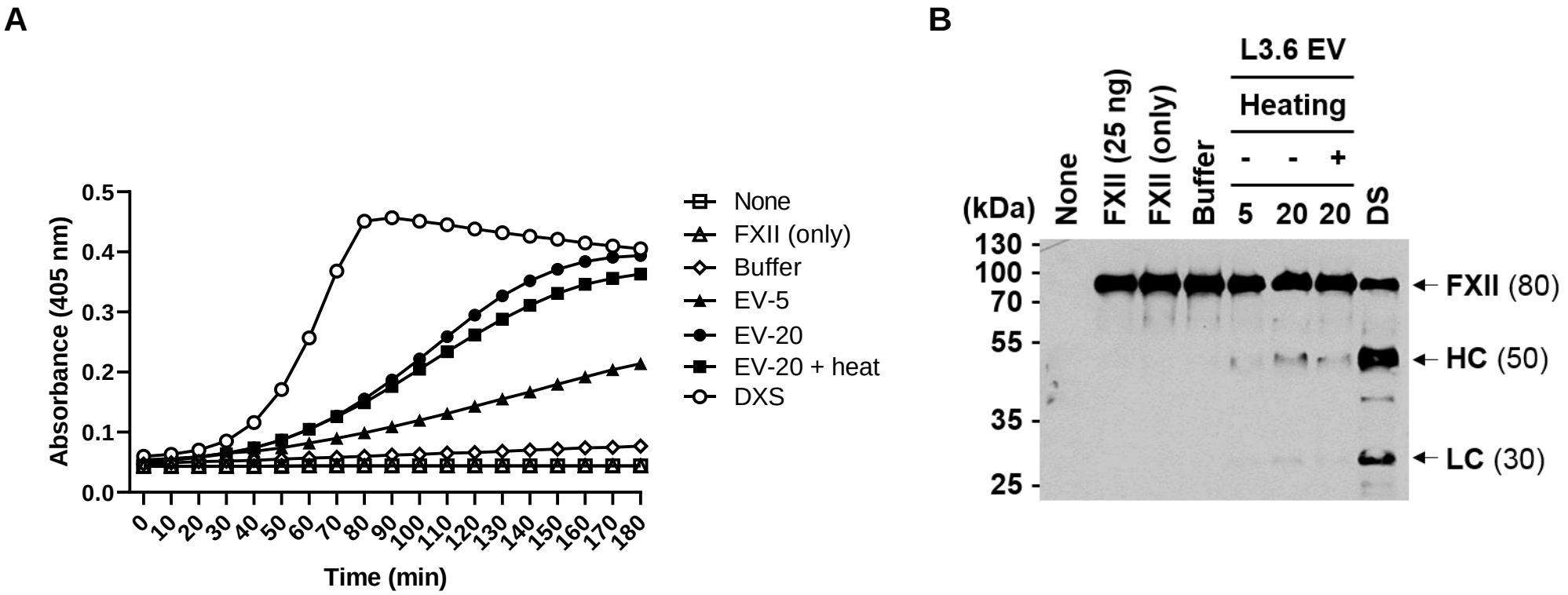

Figure S4. Effect of heating on FXII activation by L3.6 EV. (A) L3.6 EV were incubated at 25°C or 70°C for 10 minutes before addition to reaction buffer containing S-2302 chromogenic substrate. Substrate hydrolysis was then monitored at 405 nm for 180 minutes. (B) FXII cleavage was analyzed by immunoblotting in samples from reaction in (A). Purified FXII (25 ng) was used to denote the location of uncleaved FXII. Cleaved heavy chain (HC) and light chain (LC) are indicated by arrows at ~50 and ~ 30 kDa respectively.

Figure S5

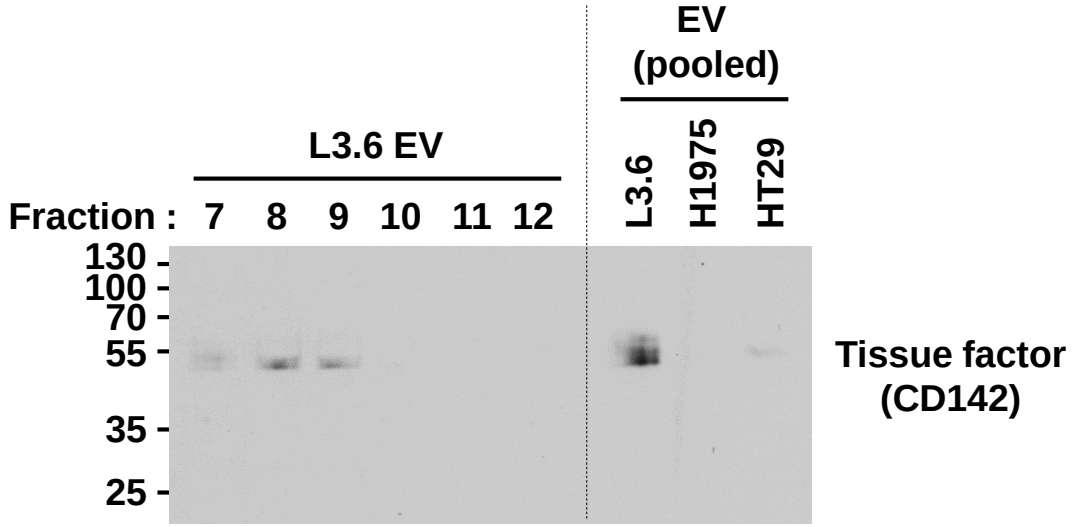

Figure S5. Tissue factor expression in cancer cell-derived EV. EV derived from cancer cell lines (L3.6, H1975, and HT29) were isolated using qEV size exclusion chromatography. Eluted fractions (7-12) from L3.6 and pooled fractions (7-9) from L3.6, H1975, and HT29 were concentrated to 100 microliters and 50 microliters of this solution was analyzed for tissue factor by immunoblotting using rabbit monoclonal anti-human tissue factor antibody (Cell Signaling Technology, # 97438S).