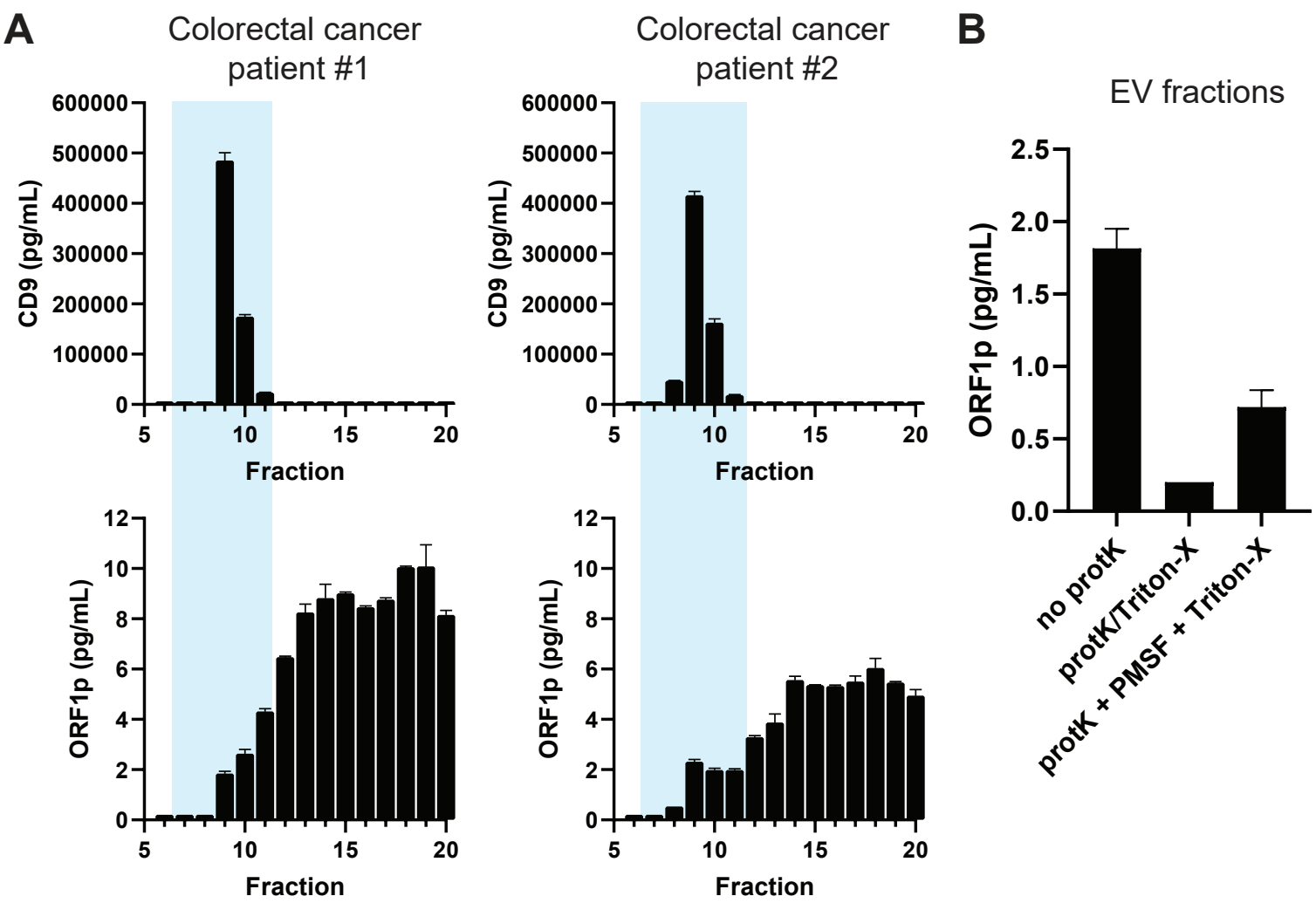

Supplementary Figure 6. Analysis of circulating ORF1p levels in extracellular vesicles (EVs) fractions of colorectal cancer patient plasma. 500 µL plasma was filtered through a 0.45 µm centrifugal filter and fractionated with a Sepharose CL-6B resin packed size exclusion chromatography column (A) CD9 and ORF1p levels in each SEC fraction as measured by Simoa. Blue highlighted boxes denote extracellular vesicle (EV)-containing fractions. The majority of circulating ORF1p is measured in free protein fractions. (B) Determination of ORF1p levels inside EVs via proteinase K (protK) protection assays. EV-containing fractions were pooled and concentrated and treated with proteinase K, followed by the serine protease inhibitor phenylmethylsulfonyl fluoride (PMSF) and 0.5% Triton-X. Controls without proteinase K digestion (no protK) and with simultaneous proteinase K and Triton-X treatment (protK/Triton-X) were performed. Decreased ORF1p concentrations were measured after proteinase K digestion and subsequent EV lysis by Triton-X, further suggesting that only a very small amount of circulating ORF1p is in EVs.