



**Supplementary Figure 16. Screening of multimeric nanobodies for ORF1p detection with Simoa.** (A) Signal-to-background (Signal:Bg) comparisons of multimeric nanobodies as detector reagents on the Simoa platform, using recombinant ORF1p protein. A four-plex assay format, with a unique fluorescent dye-encoded bead type for each of the four capture reagents tested, was used for screening. (B) Signal-to-background comparison of multimeric nanobodies as capture reagents on Simoa, using recombinant ORF1p protein, paired with the monoclonal antibody Ab5 as detector reagent. Sub-labels “A” and “B” refer to different nanobody concentrations used during conjugation to beads. (C-D) Screening of select affinity reagent pairs in small sets of plasma samples from healthy and cancer (colorectal and gastroesophageal) patients. Each assay is denoted by the capture/detector reagent pair. The first-generation assay (Nb5/Ab6) measurements are depicted for comparison. All assays were performed as three-step Simoa assays unless otherwise indicated (two-step). Blue dashed lines indicate the assay limits of detection, accounting for four-fold dilution.