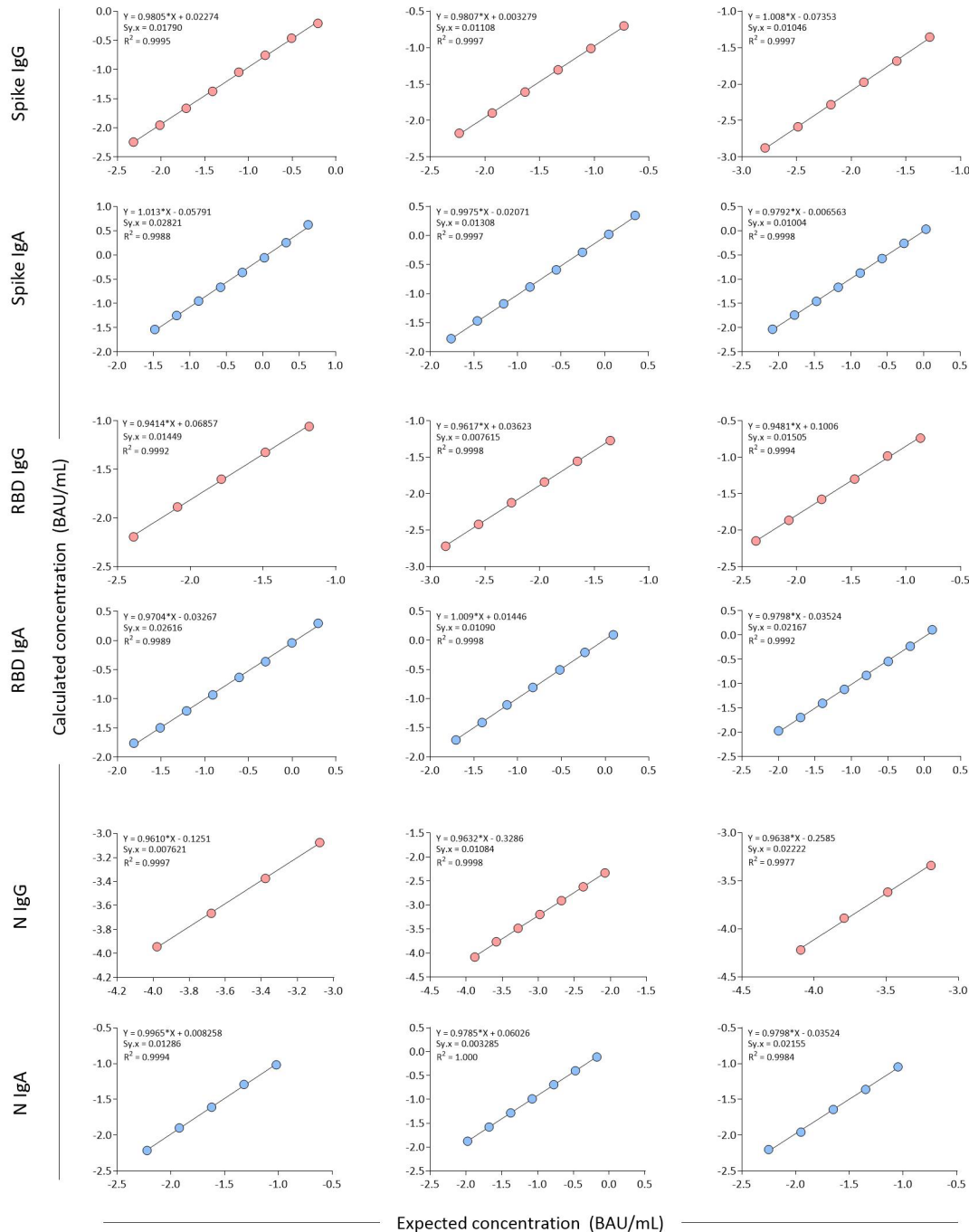
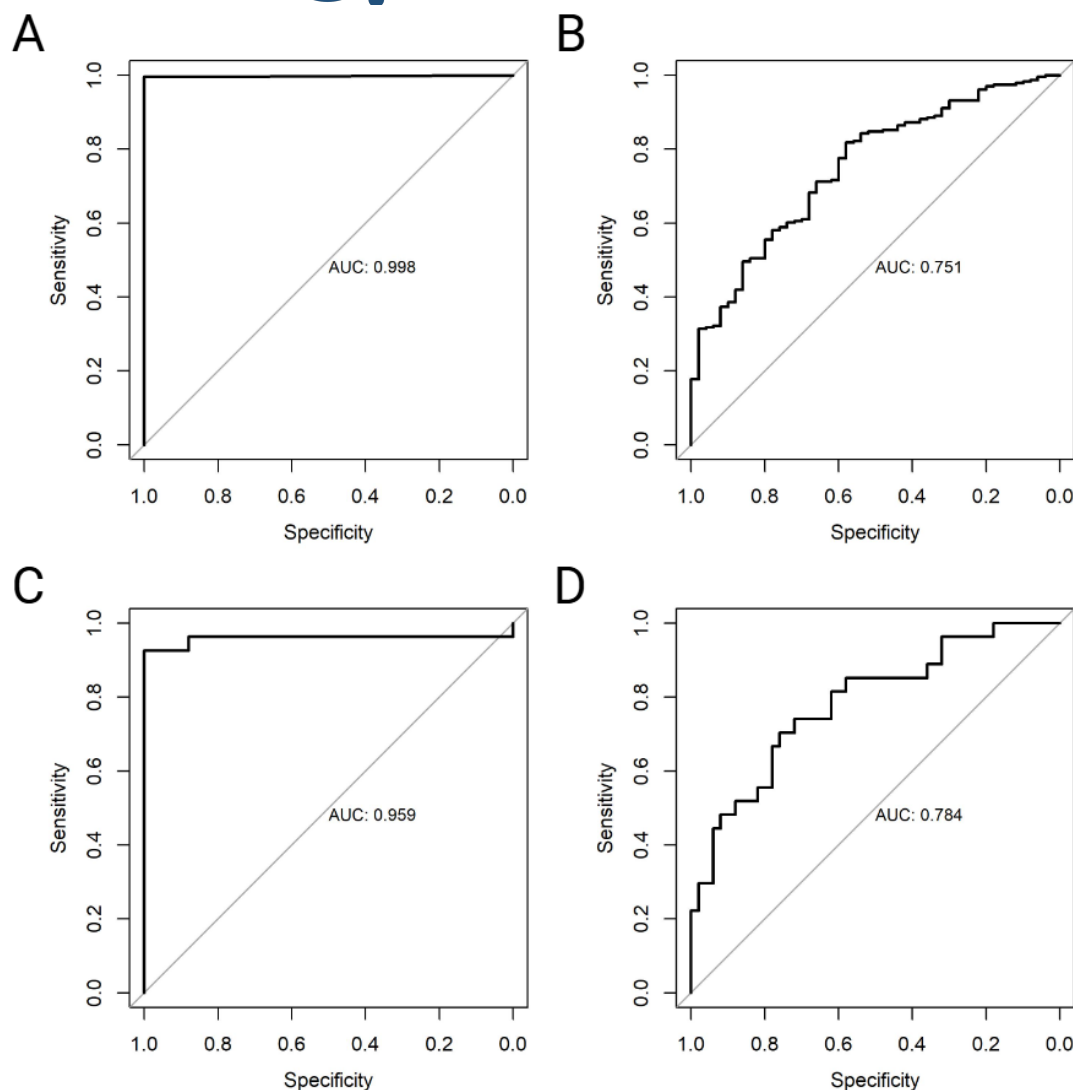




Supplemental Figure 1. Dilutional linearity of breast milk samples. SARS-CoV-2 binding antibody concentrations (BAU/mL) were measured in serially diluted human breast milk (HBM) in three samples (represented by columns). Data represent linear regression analyses of spike, spike receptor binding domain (RBD), and nucleocapsid protein (N) immunoglobulin (Ig) G and IgA calculated concentrations vs. expected concentrations (both log-transformed). The linear regression equations, $Sy.x$, and R^2 are indicated.



Supplemental Figure 2. Establishment of cutoff values for SARS-CoV-2 Spike- and Nucleocapsid (N)-specific IgG and IgA positivity in human breast milk (HBM). Spike and N IgG and IgA titers were determined by the optimized ECLIA. Positivity thresholds were established by applying Receiver Operating Characteristics (ROC) and Area Under the ROC curve analysis of log-transformed binding antibodies concentrations. **(A, B)** Spike IgG and IgA thresholds were identified by comparing pre-pandemic HBM (n=50) and post-pandemic HBM from women who were vaccinated with or without self-reported infection (n=236). **(C, D)** Nucleocapsid IgA and IgG thresholds were established by comparing pre-pandemic HBM (n=50) against post-pandemic samples from vaccinated women with self-reported COVID-19 infections prior to breast milk collection (n=54).