

Fig. S1 High-throughput fluorescent neutralization assay (NA) validation. a) Neutralization titers of 51 pre-pandemic samples (negative control) and 175 serum samples of PCR-confirmed COVID-19 patients from the Massachusetts General Brigham (MGB) Biobank. The data for the PCR-confirmed samples are further separated by the duration between date of PCR-positive test and date of sample collection. Pre-pandemic samples had almost no neutralizing activity, with 1 of 51 samples having an ID<sub>50</sub> just above the cutoff of 40 (> 98.0% specificity). In contrast, 85.5% of PCR-positive samples had ID<sub>50</sub> values greater than 40; b) Replicate-to-replicate and day-to-day variability of the NA assay. Samples from the validation set (n = 418) were tested in duplicate in the same batch on the same day to evaluate replicate-to-replicate variability (top two panels) and on two separate days to assess day-to-day variability (bottom panel). Each dot represents a single sample. Correlation analysis was performed on log-transformed values. The solid lines denote complete correlation, and the black dotted lines denote a two-fold change in either direction.