Supplemental Methods

Erythrocyte preparation

Informed written consent for sample use was obtained from all donors in accordance with the Institutional Review Board of Johns Hopkins. Blood was collected by venipuncture in EDTA tubes. Participants with type O positive red blood cells were selected.

Erythrocytes were washed three times with phosphate-buffered saline (1x PBS pH 7.4) and were prepared to a hematocrit of 25% in GVB^0MgEGTA. GVB^0MgEGTA was prepared by mixing GVB^0 (gelatin veronal buffer) (Complement Technology, Inc) and 100 mM MgEGTA (Complement Technology, Inc) in a 9:1 ratio then adjusted to pH 6.4.

Serum Preparation

Normal human serum was acidified with 0.2N HCl (aNHS; Complement Technology, Inc). Recombinant protein SARS-CoV-2 spike protein subunit 1 (S1) (receptor-binding domain; RayBiotech) was added to aNHS at concentrations of 5, 10, 20 µg/ml and incubated for 15 minutes on ice.

Erythrocyte Lysis

In a 96-well V-bottom plate, a final concentration of 40% aNHS with and without S1 was added to the erythrocytes (5×10^7 cells/well) in GVB^0MgEGTA (pH 6.4) to reach a final volume of 100 µl. 0.5mM EDTA was added to aNHS as a negative control. Samples were incubated at 37°C for 1 hour, followed by centrifugation and collection of the supernatants.

Absorbance at 405 nm was measured in the cell free supernatants in a 96-well flat bottom plate using a plate reader (ELX808; BioTeK). The sample absorbance value was normalized by subtracting the absorbance of a blank sample containing erythrocytes in GVB^0MgEGTA. Hemolysis in each sample was compared to water-induced lysis of the corresponding red blood

cells, which represents 100% cell lysis. Percent hemolysis was calculated using [($OD405_{sample}$-$OD405_{blank}$)/($OD405_{water}$-$OD405_{blank}$) *100%].

Each sample was performed in duplicate and each experiment was repeated two times.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 6 (GraphPad, Inc, San Diego, CA). Data are presented as mean ± standard error of the mean (SEM) of duplicate wells.