SUPPLEMENTARY INFORMATION

Methylene Blue Is a Nonspecific Protein-Protein Interaction Inhibitor with Potential for Repurposing as an Antiviral for COVID-19

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Supplementary Figures

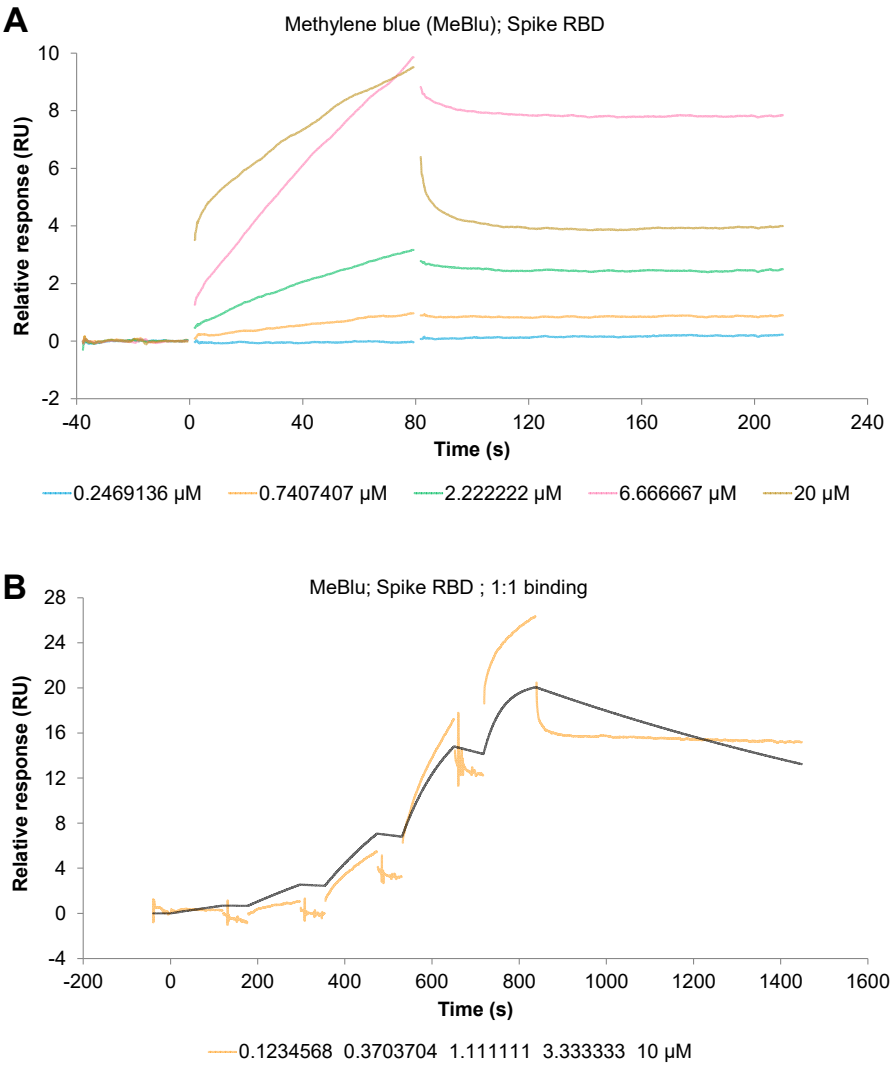

Figure S1. Binding of MeBlu to SARS-CoV-2 spike RBD as assessed via surface plasmon resonance (SPR). Multi- (**A**) and single- (**B**) cycle kinetics were used to measure binding to the SARS-CoV-2 spike RBD using a Biacore 8K instrument with a series S CM5 sensor chip (Cytiva) as described in the Methods. Initially a multi-cycle kinetic measurement was performed, however, the signals clearly did not return to baseline over the dissociation period and a regeneration condition was not established to ensure removal of all previously bound molecule before the injection of the next concentration. As a result, there were a decreasing number of binding sites available over the course of the measurement as sites became blocked by the preceding concentration(s), which meant this approach was not appropriate to use for a quantitative analysis even though qualitatively it was clear that binding was occurring. To deal with the apparent slow dissociation (signal not returning to baseline between injections) a single cycle kinetic approach was used instead. Data indicate low-micromolar binding; however, the binding behavior could

not be described well with a 1:1 binding interactions, thus preventing a quantitative analysis to determine the on/off-rate and/or KD values. Figure B shows an example of this deviation from 1:1 binding where the colored (yellow) curve is the measured data while the black curve is the fit curve produced using a 1:1 binding model. Instead, the SPR data provides qualitative support that MeBlu interacts with SARS-CoV-2 spike RBD.