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MUTATION OF A CONSERVED, HYDROPHOBIC, CRYPTIC EPITOPE IMPROVES MANUFACTURABILITY AND IMMUNOGENICITY OF THE SARS-CoV-2 RBD

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The supply of COVID-19 vaccine doses still lags behind the global demand for first time vaccination and booster doses. Distribution of vaccine doses has been far from equitable across the world given the steep prices and logistical challenges that low- and middle-income countries face. Subunit protein vaccine candidates have now been shown to elicit protective responses against SARS-CoV-2 infection, while providing additional benefits for manufacturing capability and stability requirements compared to many currently approved vaccines. Here we report a second-generation engineered RBD sequence variant with enhanced manufacturability and immunogenicity over the wild-type ancestral RBD and a first-generation engineered variant (RBD-L452K-F490W (RBD-J)). Introducing two additional mutations, S383D and L518D, to a hydrophobic cryptic epitope in the RBD core improved expression titers and biophysical stability compared to RBD-J. These two additional mutations in RBD-S383D-L452K-F490W-L518D (RBD-J6) ablated the interaction of two neutralizing antibodies, CR3022 and EY6A, targeting the class 4 epitope on the RBD core, but the protein is still bound by human convalescent sera. Mice immunized with a Beta sequence variant of RBD-J and RBD-J6 displayed on a virus-like particle were protected against challenges with Alpha and Beta variants of SARS-CoV-2. Sera from mice immunized with three doses of a RBD-J6 β – VLP showed comparable neutralizing activity to several variants of concern compared to two doses of Comirnaty.

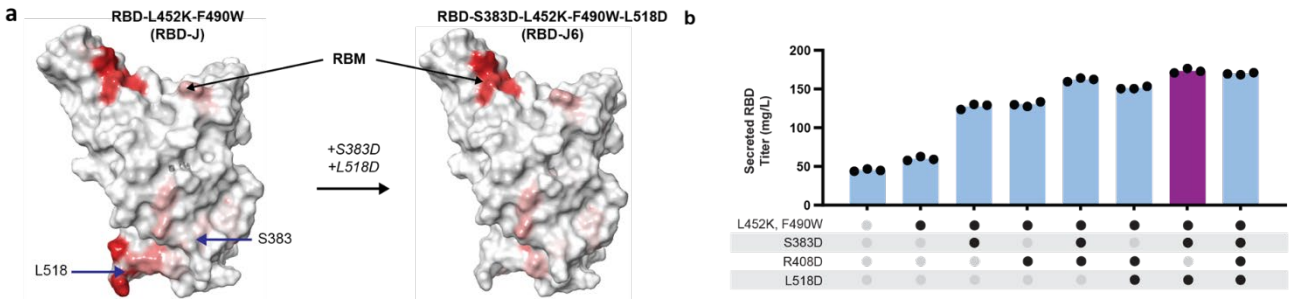

Figure 1. Mutations to hydrophobic surface on RBD core improved secreted titers of RBD.
(a) Computational analysis of RBD reveals hydrophobic patches on surface (red). Mutations to RBD core reduced surface hydrophobicity. (b) Pairwise combinations of mutations to RBD-core improve secretion titers of RBD measured by reverse phase liquid chromatography.