Next-generation rationally-designed vaccine adjuvants represent a significant breakthrough to enable development of vaccines against challenging diseases including tuberculosis, HIV, and malaria. New vaccine candidates often require maintenance of a cold-chain process to ensure long-term stability and separate vialing to enable bedside mixing of antigen and adjuvant. This presents a significant financial and technological barrier to worldwide implementation of such vaccines. Herein we describe the development of a single-vial lyophilized thermostable tuberculosis vaccine comprised of an antigen (ID93) and an oil-in water emulsion adjuvant (GLA-SE), using a design of experiment (DOE) approach. Stabilizing excipients were identified, and the effect of various factors were evaluated to determine optimized formulations that minimized GLA and ID93 degradation, particle size growth, and pH change, while optimizing cake quality. Formulations were identified that are stable at elevated temperatures. Further this vaccine retains the ability to elicit both antibody and TH1 responses against the vaccine antigen and protect against experimental challenge with Mycobacterium tuberculosis. These results represent a significant breakthrough in the development of vaccine candidates that can be implemented throughout the world without being hampered by the necessity of a continuous cold chain or separate adjuvant and antigen vials.