Built-in RNA-mediated Chaperone (Chaperna) for Antigen Folding Tailored to Immunized Hosts

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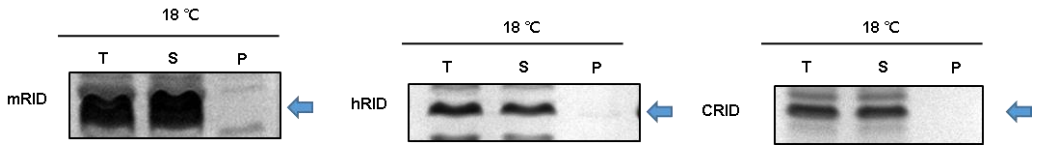
Running title: Chaperna-Mediated Antigen Folding

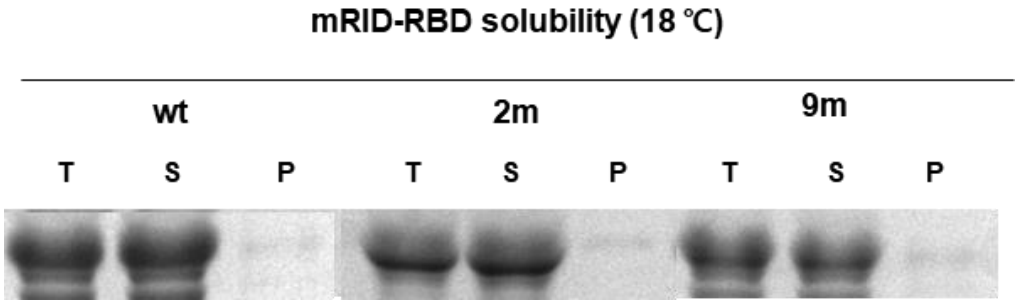
Supplementary Figures and Tables



Supplementary Figure 1. Expression levels of chaperna (mRID, hRID, and cRID) produced using an *Escherichia coli* expression system were analyzed by SDS-PAGE.

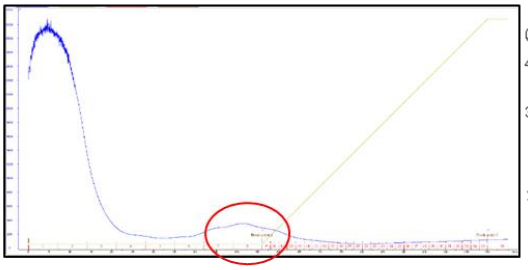
Supplementary Table 1. Point mutations sites (RNA interaction domain) of mRIDs (wild-type (wt), 2m mutant, and 9m mutant).

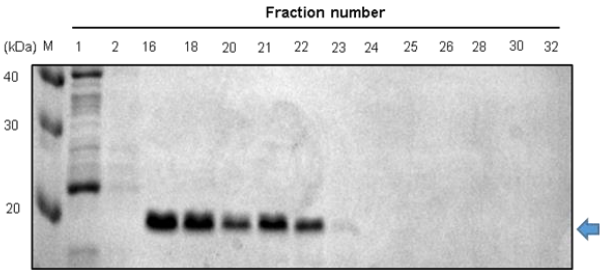
mRID(wt) amino seq	HMSEQATLQESEVKVDGEQKLSKNELKRRLKAEKKLAEKEAKQKEL SEKQLNQTASAPNHTADNGVGAEEETLDDDDDDSGENLYFQGTGSD IVDKLHHHHHH
mRID(2m) amino seq (K23A/K27A)	HMSEQATLQESEVKVDGEQKLSANELARRLKAEKKLAEKEAKQKEL SEKQLNQTASAPNHTADNGVGAEEETLDDDDDDSGENLYFQGTGSD IVDKLHHHHHH
mRID(9m) amino seq (K23A/K27A/R28A/K31A/K34A/ K35A/K39A/K42A /K44A)	HMSEQATLQESEVKVDGEQKLSANELAARLAAEAALAEAEAAQAEL SEKQLNQTASAPNHTADNGVGAEEETLDDDDDDSGENLYFQGTGSD IVDKLHHHHHH



Supplementary Figure 2. Comparison of solubilities of wild-type (wt), 2m mutant, and 9m mutant mRID-RBD molecules. All proteins were expressed at 18°C. The cell lysates were separated into total (T), soluble (S), and pellet (P) fractions by centrifugation. The solubilities of the cell extracts were determined by SDS-PAGE.

< mRID >





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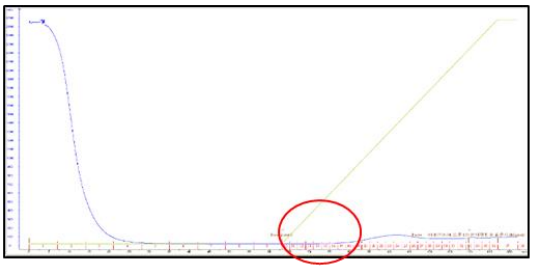
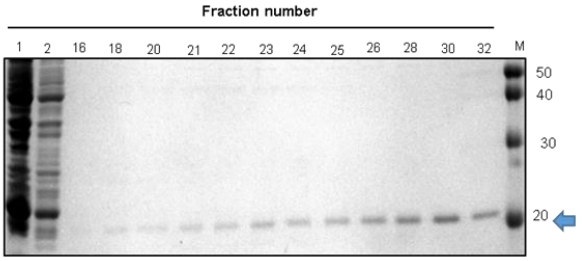
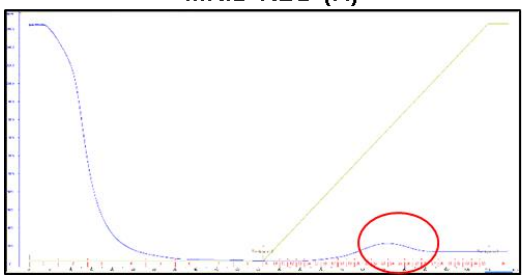
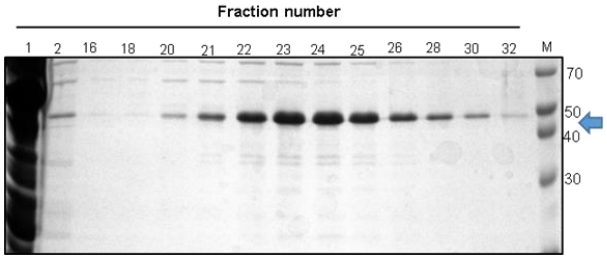




< mRID-RBD (K) >





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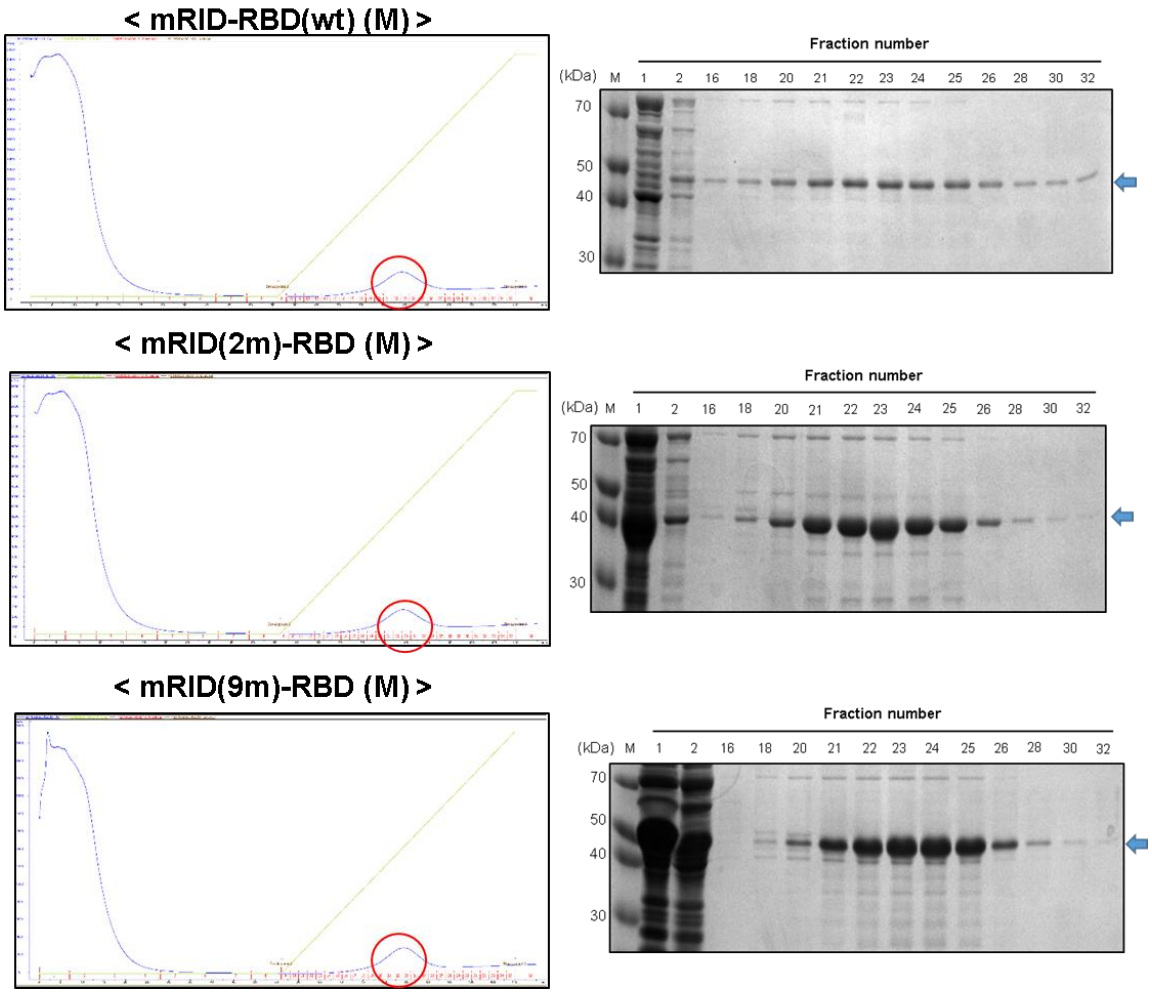
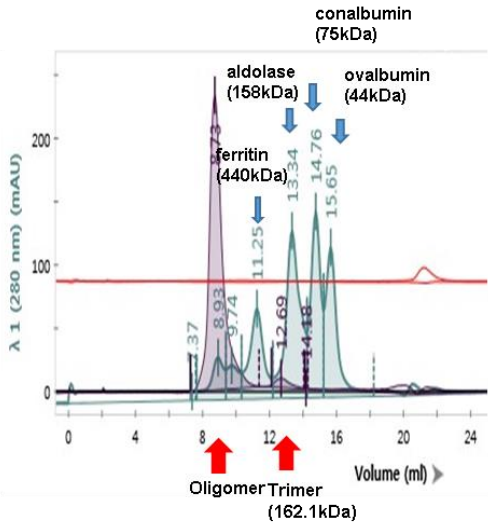
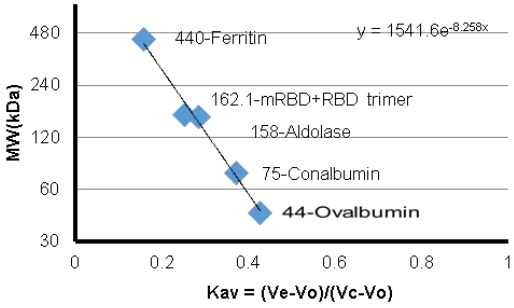
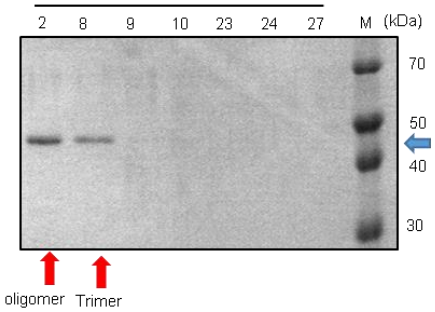


Supplementary Figure 3. Purification profile of proteins obtained by SDS-PAGE. mRID, mRID- RBD (Korea strain), mRID-HR2, mRID(wt)-RBD, mRID(2m)-RBD, and mRID(9m)-RBD were purified using Ni–NTA resin. The blue arrows indicate the purified target proteins.

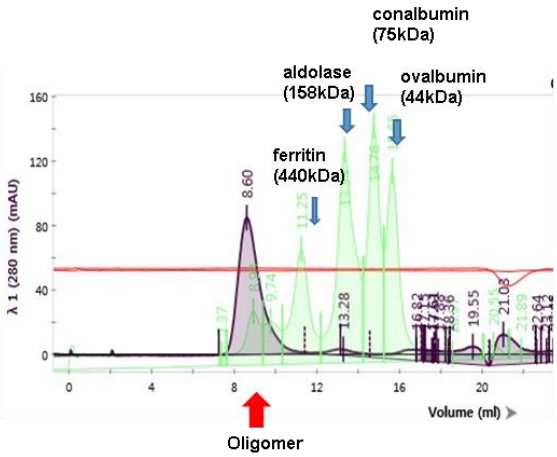
< mRID(wt)-RBD >

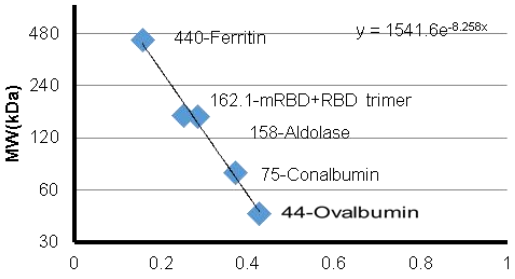


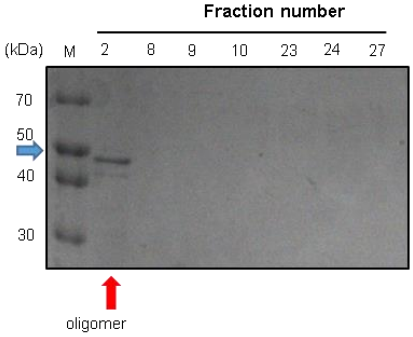




< mRID(9m)-RBD >







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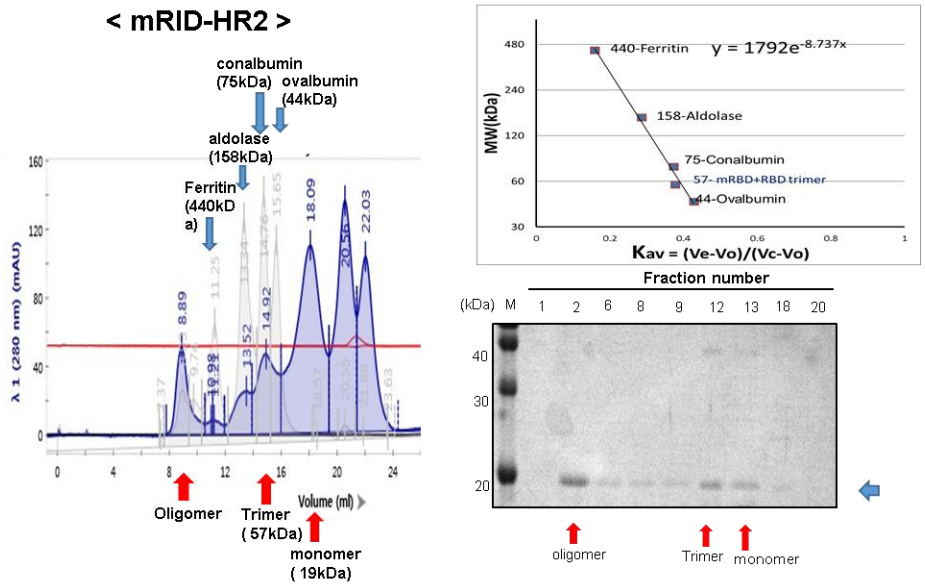
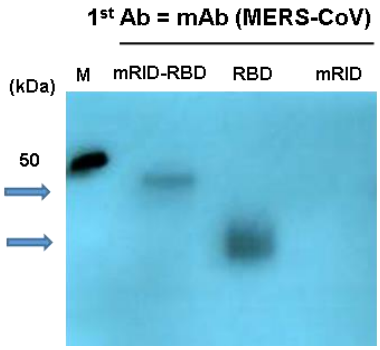


Supplementary Figure 4. Size exclusion chromatography (SEC) profiles of mRID-fused proteins. Separation of purified mRID-RBD (wild-type (wt) and 9m mutant) and mRID-HR2 into oligomers, trimers, or monomers by SEC. The separated proteins were analyzed by SDS-PAGE. The protein sizes were determined using a high molecular weight (HMW) Gel Filtration Calibration Kit and a distribution coefficient (Kav) graph. The blue arrows indicate the purified target proteins.



Supplementary Figure 5. The binding of mAb29 to the RBD was assessed by western blot analysis. The blue arrows represent the target proteins. All proteins were purified using a Ni–NTA column.