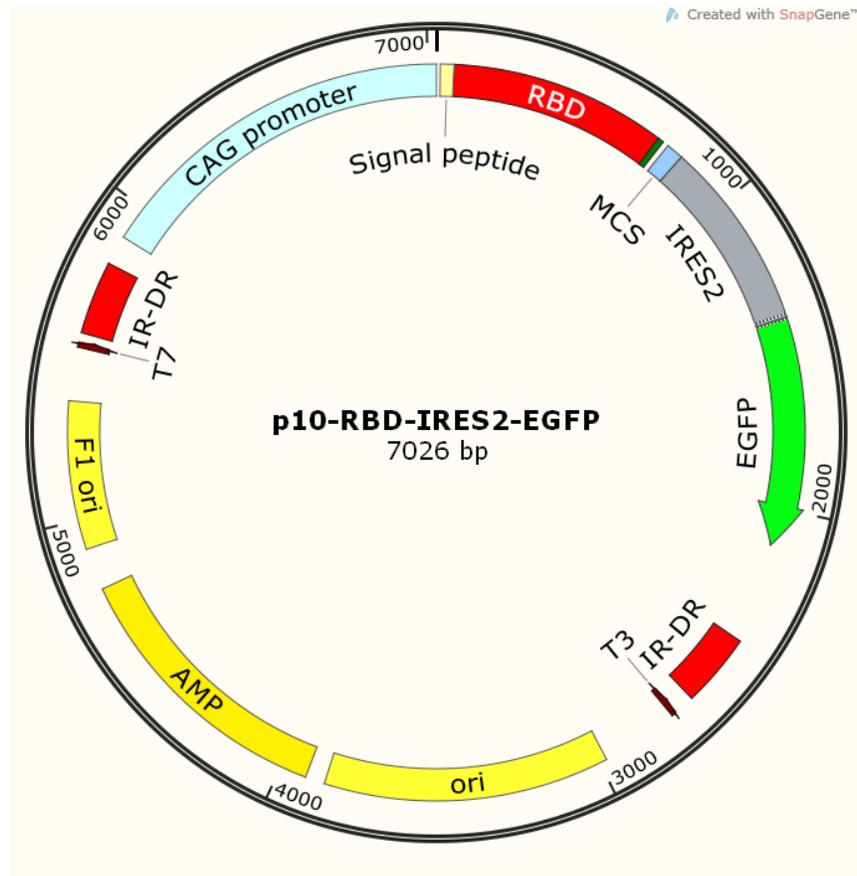
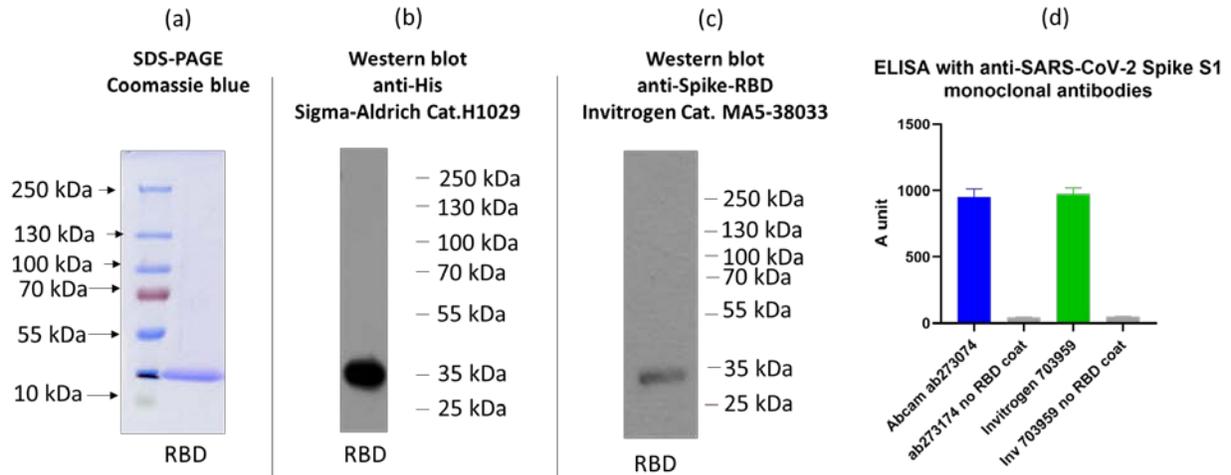


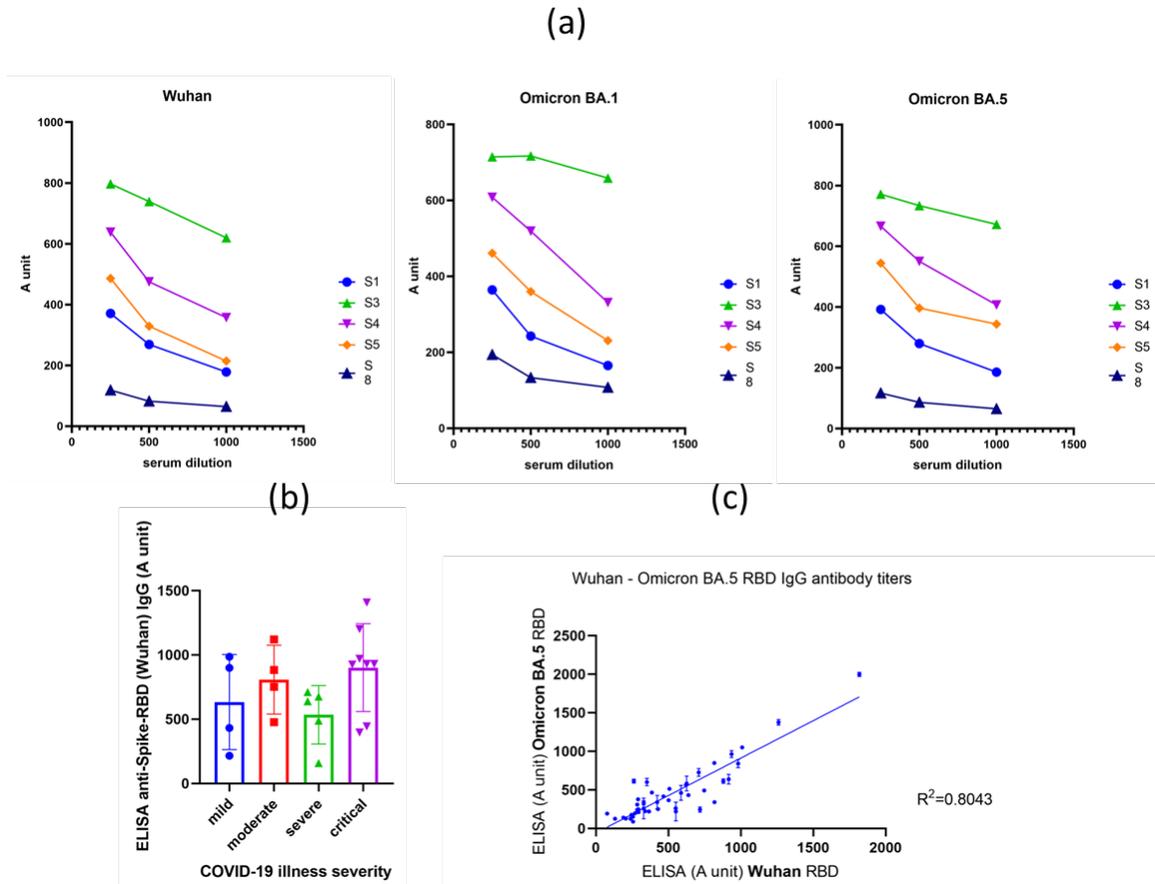
Supplementary materials for Mózner et al. “Application of an RBD-based simple immunoassay for assessing humoral immunity against emerging SARS-CoV-2 virus variants”



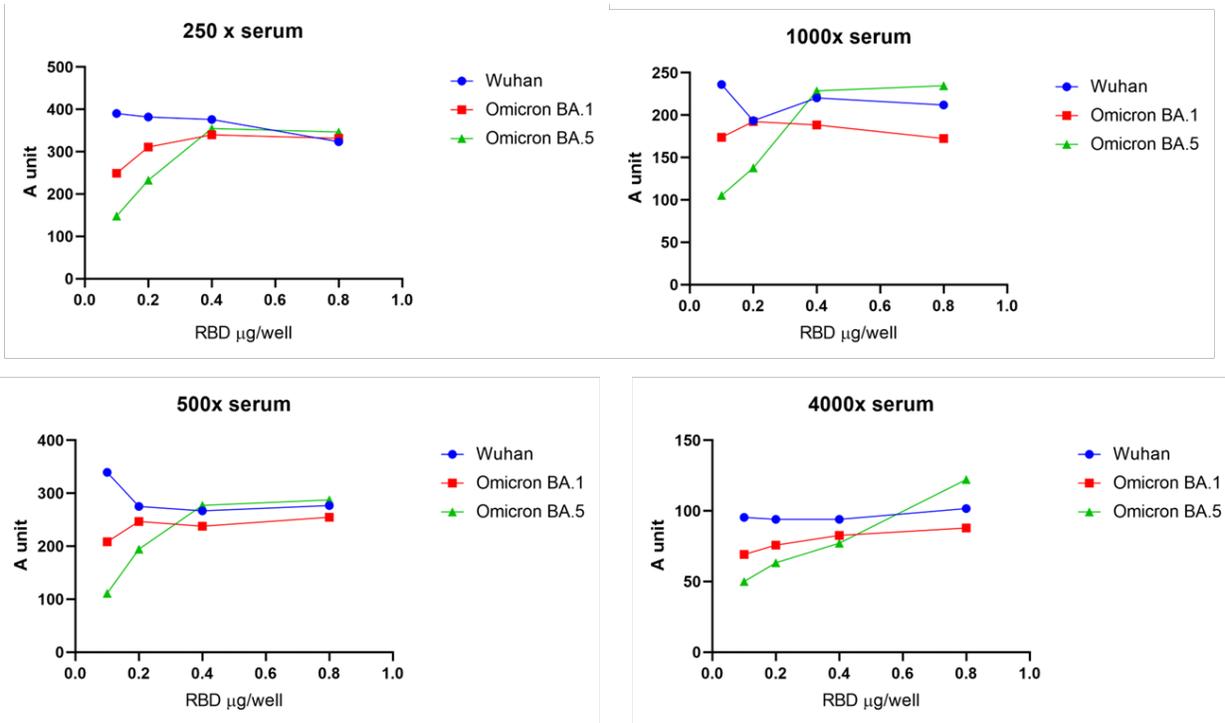
Suppl. Figure 1. The p10-RBD-IRES2-EGFP transposon vector map



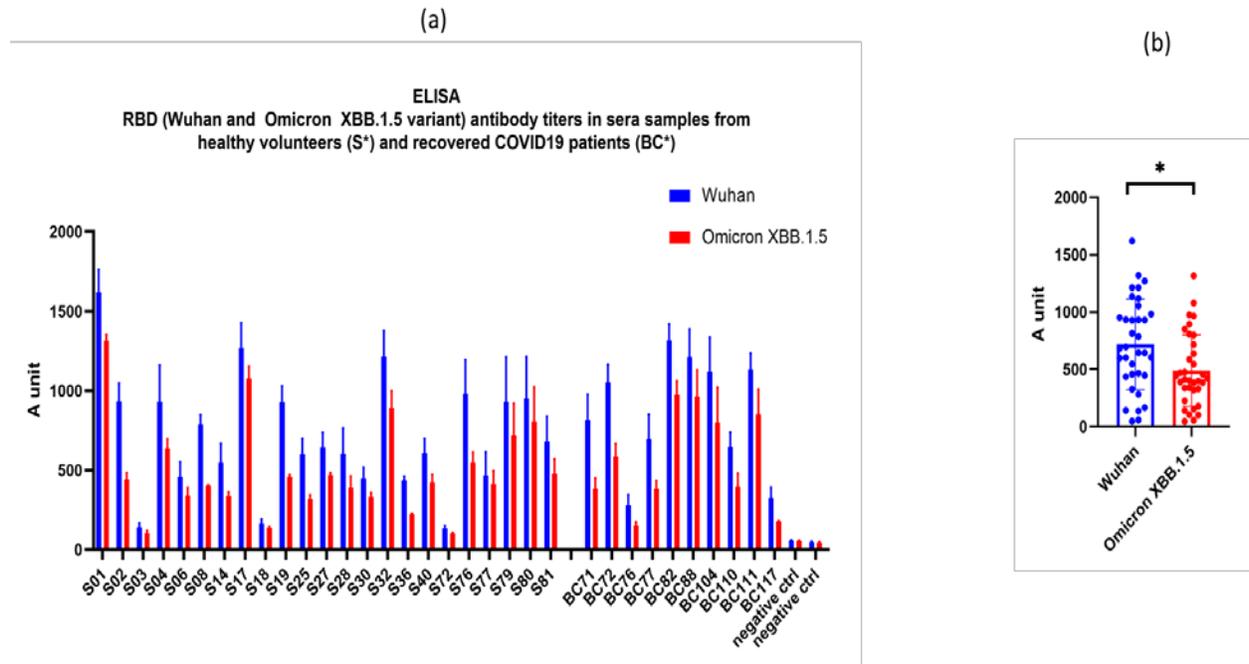
**Suppl. Figure 2.** The RBD production was examined by SDS-polyacrylamide gel electrophoreses and Western blotting. (a) Shows the purified RBD protein after SDS-PAGE and Coomassie blue staining. (b) Shows the purified RBD protein after SDS-PAGE and western blot using the anti-His primary antibody (Sigma-Aldrich Cat.H1029). (c) Shows the purified RBD protein after SDS-PAGE and western blot using the anti-RBD primary antibody (Invitrogen Cat. MA5-38033). (d) ELISA results of RBD(Wuhan) detected by two anti-SARS-CoV-2 Spike S1 commercially available monoclonal antibodies (Abcam Cat. ab273074 and Invitrogen Cat. 703959), controls: ELISA coating without RBD.



**Supplementary Figure 3.** (a) Anti-RBD (Wuhan, Omicron BA.1 and Omicron BA.5 RBD) IgG antibody titers - ELISA results of 5 individuals (serum dilutions 250x, 500x, 1000x) samples gathered in 11/2022. S1, S3, S4, S5 are serum samples of fully vaccinated individuals, while S8 is a sample of a non-vaccinated individual. (b) Anti-RBD (Wuhan) IgG antibody titers of 21 recovered COVID-19 patients grouped by severity of illness. (c) Linear regression of IgG antibody titers against Wuhan and Omicron BA.5 RBDs measured in volunteers.



**Supplementary Figure 4.** ELISA measurement with different RBD protein amounts and serum dilutions of a fully vaccinated individual, sample gathered in 11/2022. Absorbance measured at 660 nm 10 minutes after addition of TMB substrate.



**Supplementary Figure 5.** ELISA measurement. (a) Anti-RBD (Wuhan and Omicron XBB.1.5) antibody titers in vaccinated (S\*) and recovered individuals (BC\*). (b) Summary: antibody titer means of anti-Wuhan-RBD and anti-Omicron XBB.1.5.

**Supplementary data:**

**RBD (Wuhan) protein sequence (319-541 of SARS-CoV-2 Spike):**

MFVFLVLLPLVSSQRVQPTESIVRFPNITNLCPPGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPT  
KLNDLCFTNVYADSFVIRGDEVRQIAPGQQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGNYNYLYRLFRKSNLKPFE  
ERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFHH  
HHHH\*

**RBD (Omicron BA.1)**

MFVFLVLLPLVSSQRVQPTESIVRFPNITNLCPPDEVFNATRFASVYAWNRKRISNCVADYSVLYNLPFFTFKCYGVSPT  
KLNDLCFTNVYADSFVIRGDEVRQIAPGQQTGNIADYNYKLPDDFTGCVIAWNSNKLDSKVSIGNYNYLYRLFRKSNLKPFE  
RDISTEIQAGNKPENGVAGFNCYFPLRSYFRPTYGVGHQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFHHH  
HHH\*

**RBD (Omicron BA.5 – same as Omicron BA.4)**

MFVFLVLLPLVSSQRVQPTESIVRFPNITNLCPPDEVFNATRFASVYAWNRKRISNCVADYSVLYNLPFFAFKCYGVSPT  
KLNDLCFTNVYADSFVIRGNEVSIAPGQQTGNIADYNYKLPDDFTGCVIAWNSNKLDSKVGNYNYLYRLFRKSNLKPFE  
ERDISTEIQAGNKPENGVAGFNCYFPLQSYGFRPTYGVGHQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFHH  
HHHH\*

**RBD (Omicron XBB.1.5)**

MFVFLVLLPLVSSQRVQPTESIVRFPNITNLCPPDEVFNATRFASVYAWNRKRISNCVADYSVLYNLPFFAFKCYGVSPT  
KLNDLCFTNVYADSFVIRGNEVSIAPGQQTGNIADYNYKLPDDFTGCVIAWNSNKLDSKPSIGNYNYLYRLFRKSKLPFE  
RDISTEIQAGNKPENGVAGFPCYSPQSYGFRPTYGVGHQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFHHH  
HHH\*