

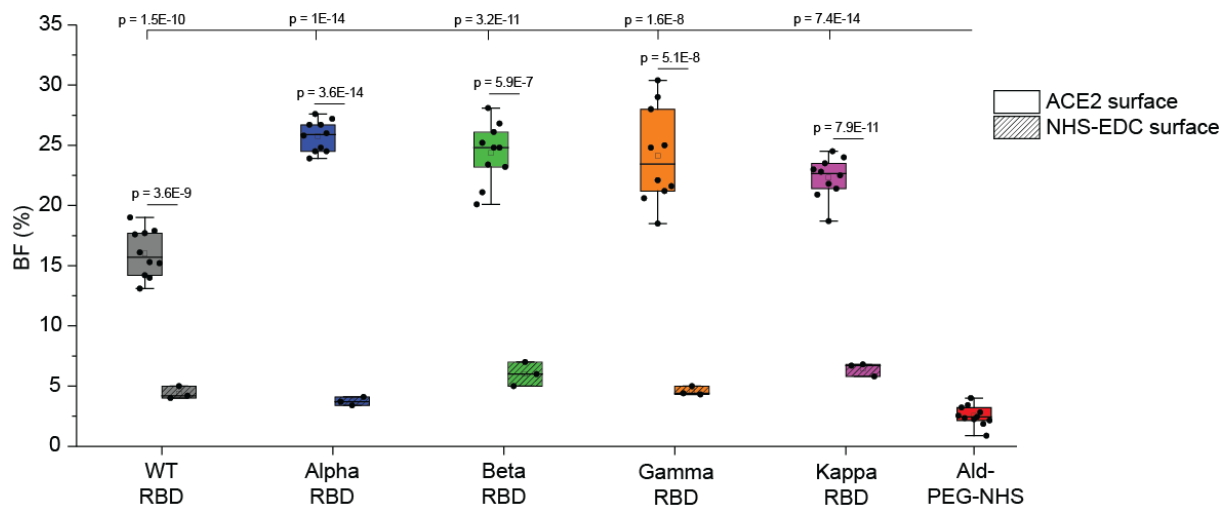
Supplementary Information for

**Understanding the structural, energetics and neutralization basis of
SARS-CoV-2 variants using atomic force microscopy.**

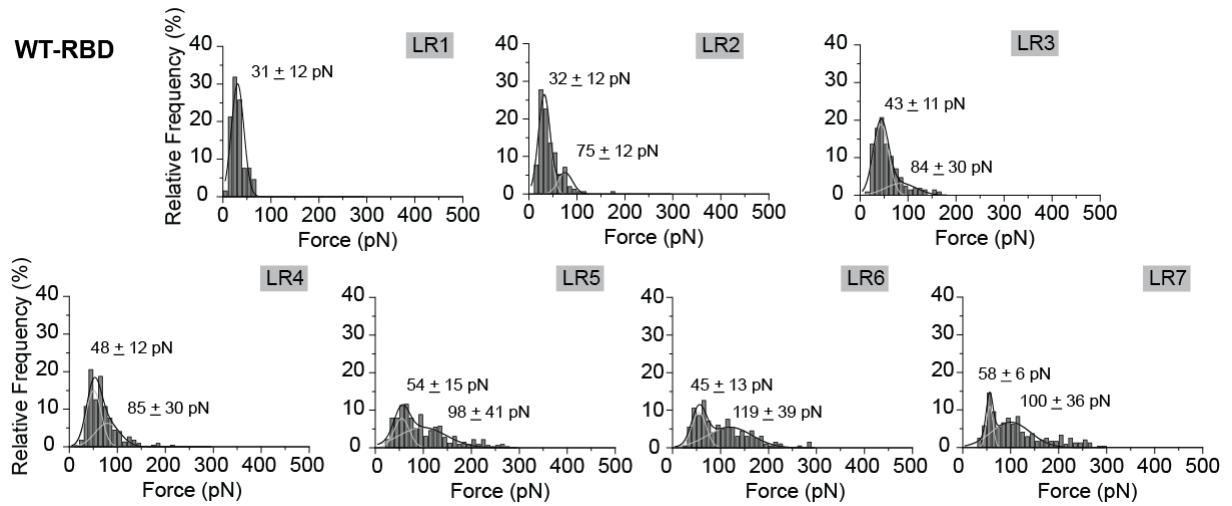
Koehler *et al.*

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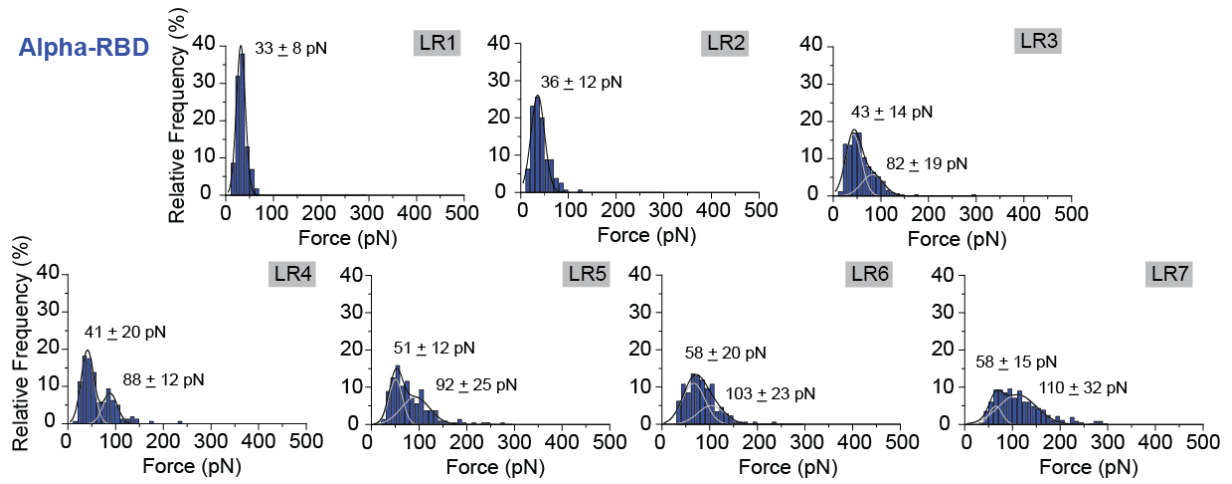
- Supplementary Figures 1 to 6



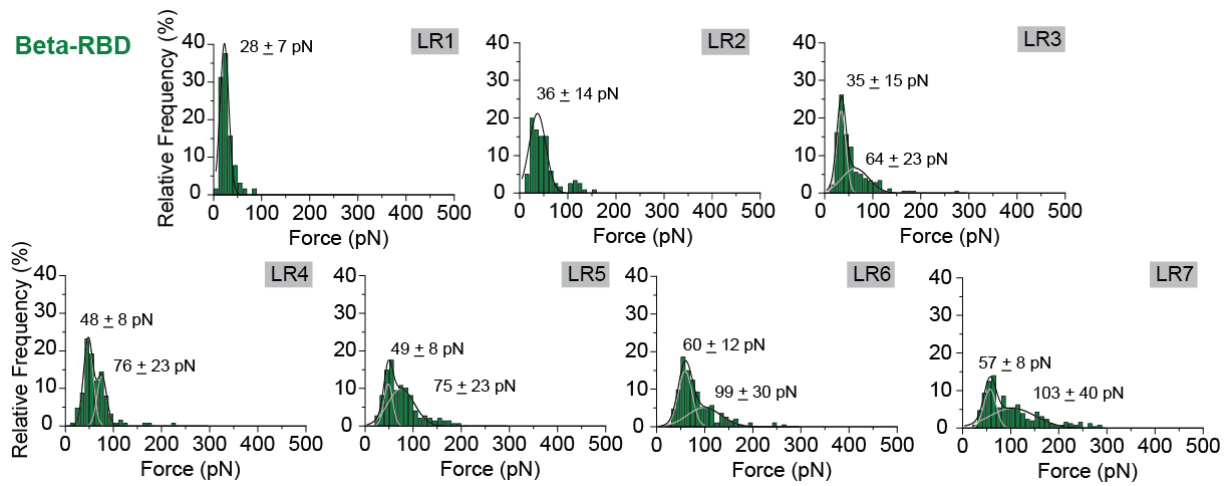
Supplementary Figure 1 | RBD mutants specifically interact with ACE2 receptor. Box plot of specific binding frequencies (BF) measured by AFM between the functionalized tip (RBD mutants) and the grafted ACE2 model surface or the NHS-EDC surface, lacking ACE2 receptor (dashed boxes). One data point belongs to the BF from one map acquired at 1 $\mu\text{m/s}$ retraction speed and 250 ms contact time. The square in the box indicates the mean, the colored box the 25th and 75th percentiles, and the whiskers the highest and lowest values of the results. The line in the box indicates the median. N = 10 maps examined over 3 independent experiments for the ACE2 surface. N = 3 maps examined over 1-2 independent experiments for the NHS-EDC surface. P-values were determined by two-sample t test in Origin.



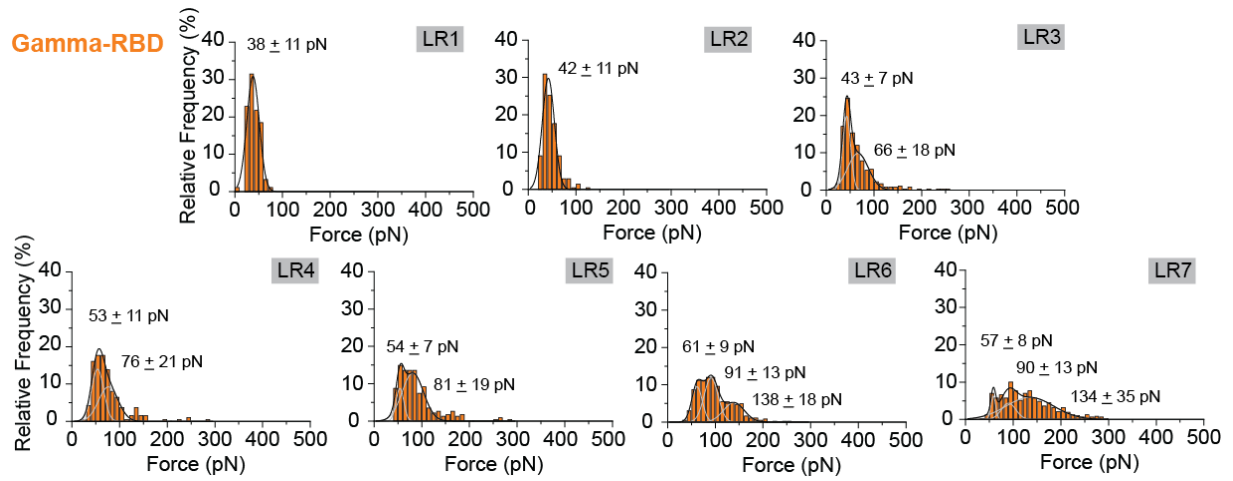
Supplementary Figure 2 | Probing WT-RBD binding to ACE2 model surfaces. Force and LR were extracted from force-distance curves and sorted in narrow LR ranges (LR1-LR7). The rupture forces for each LR range were plotted as histograms and fitted with multipeak Gaussian fits. The maxima of all force peaks are indicated. N = 1435 from 4 independent experiments.



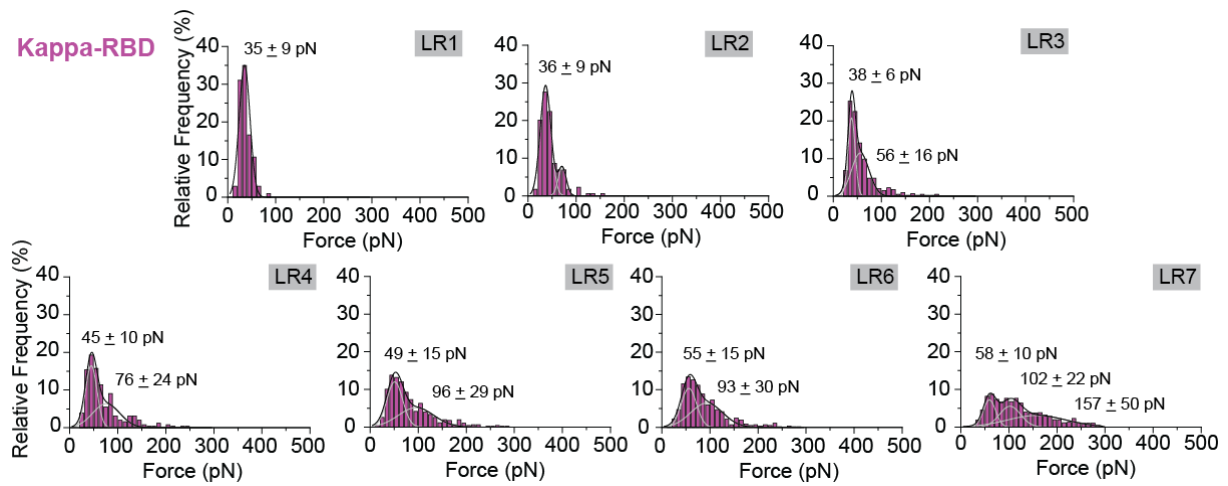
Supplementary Figure 3 | Probing Alpha-RBD binding to ACE2 model surfaces. Force and LR were extracted from force-distance curves and sorted in narrow LR ranges (LR1-LR7). The rupture forces for each LR range were plotted as histograms and fitted with multi-peak Gaussian fits. The maxima of all force peaks are indicated. N = 1542 from 3 independent experiments.



Supplementary Figure 4 | Probing Beta-RBD binding to ACE2 model surfaces. Force and LR were extracted from force-distance curves and sorted in narrow LR ranges (LR1-LR7). The rupture forces for each LR range were plotted as histograms and fitted with multippeak Gaussian fits. The maxima of all force peaks are indicated. N = 1095 from 3 independent experiments.



Supplementary Figure 5 | Probing Gamma-RBD binding to ACE2 model surfaces. Force and LR were extracted from force-distance curves and sorted in narrow LR ranges (LR1-LR7). The rupture forces for each LR range were plotted as histograms and fitted with multi-peak Gaussian fits. The maxima of all force peaks are indicated. N = 1953 from 4 independent experiments.



Supplementary Figure 6 | Probing Kappa-RBD binding to ACE2 model surfaces. Force and LR were extracted from force-distance curves and sorted in narrow LR ranges (LR1-LR7). The rupture forces for each LR range were plotted as histograms and fitted with multi-peak Gaussian fits. The maxima of all force peaks are indicated. N = 2720 from 4 independent experiments.