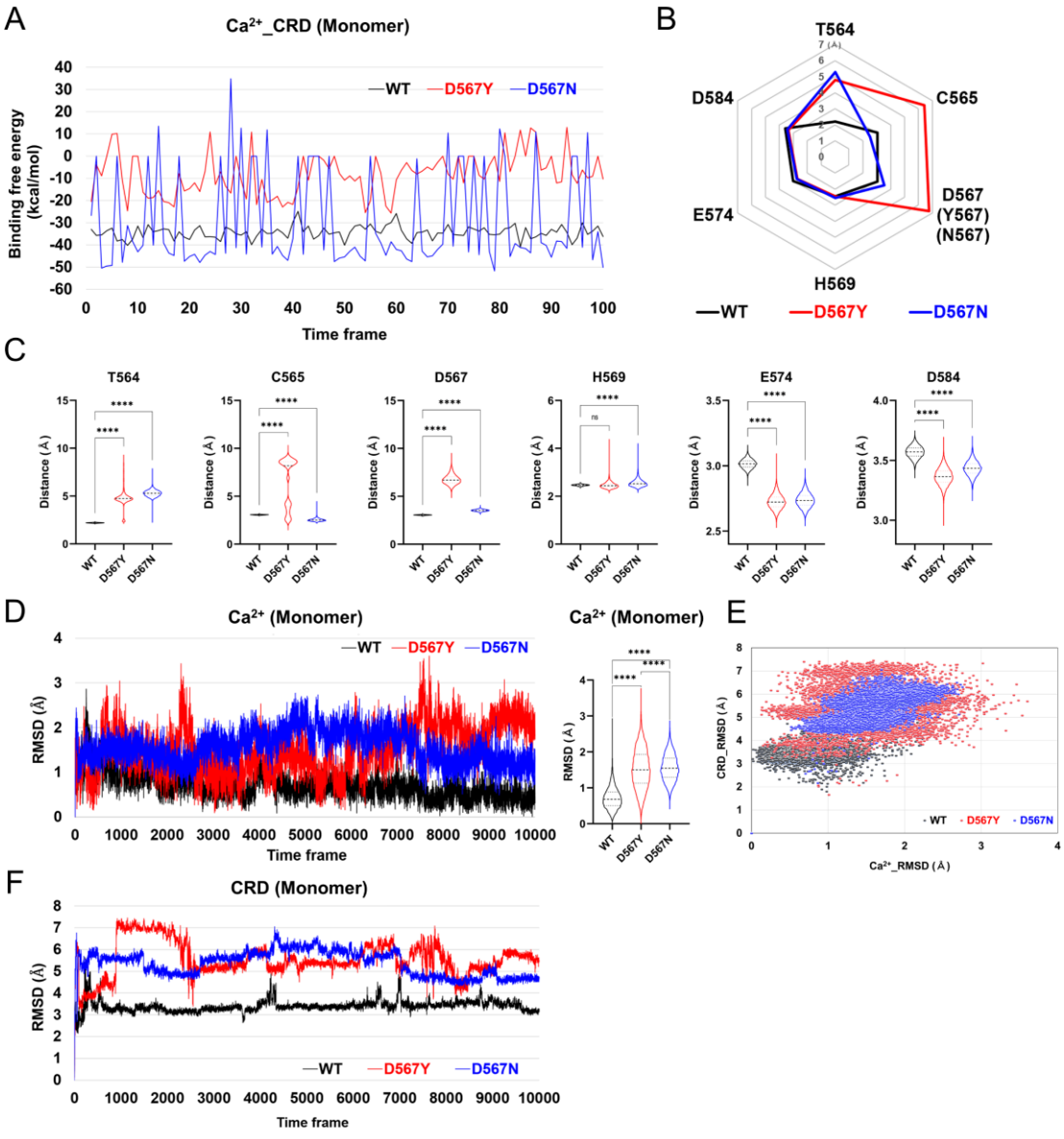
Supplementary Figure S2



Supplementary Figure S2. 3D fluctuation of the CRD caused by CaLM mutation

A, Binding free energy calculated every 10 ns during a 1 μs simulation. The three lines indicate temporal changes in binding free energy with the Ca^{2+} ion of wild-type (black), D567Y (red), and D567N (blue) RET-CRDs in each time frame of 10 ns.

B, Radar plot showing the average distance between Ca^{2+} and amino acid residues of CaLM

in wild-type (black line), D567Y (red line), and D567N (blue line) during simulation of CRD monomers. The distances between the Ca^{2+} ion and the carboxyl carbon atom of the negatively charged side chains or the oxygen atom of the backbone of amino acids contributing to coordinated binding were calculated. Calculation at amino acid reside 567 was performed for aspartic acid (wild-type), asparagine (D567N), and tyrosine (D567Y). The distances between Ca^{2+} ions and the Cγ atom in the D567N mutant and the aromatic ring center of the side chain in the D567Y mutant were calculated. Each number represents the average of the distances in Å calculated every 100 ps during the MD simulation time.

C, Violin plots showing the distance between the Ca^{2+} ion and amino acid residues contributing to coordinated bond formation (T564, C565, D567, H569, E574, and D584) calculated every 100 ps in the MD simulation. The width of each strip is proportional to the relative minimum distance frequency. A black dashed line represents the median of the minimum distance. Each dotted line represents the interval between the end of the first quartile and the beginning of the fourth quartile. Asterisks indicate significantly different distributions (n.s.: not significant, ****: $P < 0.0001$; one-way ANOVA with Tukey's multiple comparisons test).

D, RMSD values of the Ca^{2+} ion with respect to the location of the initial coordinate in wild-type and D567 mutants. *Left*, the three lines indicate temporal changes in wild-type (black), D567Y (red), and D567N (blue) RET-CRDs in each time frame of 100 ps. *Right*, violin plot showing the RMSD values calculated for every 100 ps time frame. A black dashed line indicates the median RMSD position. Each dotted line represents the interval between the end of the first quartile and the beginning of the fourth quartile. Asterisks indicate significantly different distributions (n.s.: not significant, ****: $P < 0.0001$; one-way ANOVA with Tukey's multiple comparisons test).

E, Scatterplot of the RMSD of the Ca^{2+} ion (x-axis) and heavy atoms of each residue of CRD monomers (y-axis) in RET wild-type (black spot), D567Y (red spot), and D567N (blue spot) during MD simulations. Each spot shows the RMSD value calculated every 100 ps during the MD simulation time.

F, RMSD values were calculated on the heavy atoms of each residue with respect to the location of the initial coordinate in wild-type and D567 mutants. The color-coding is the same as in (D).