Supporting information for manuscript titled "Computational and Experimental Characterization of Patient Derived Mutations Reveal an Unusual Mode of Regualtory Spine Assembly and Drug Sensitivity in EGFR Kinase"

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Supporting Information

Supporting Information Available: Figure S1, Figure S2, Figure S3, Figure S4, Figure S5, Figure S6, Figure S7, Figure S8, Figure S9, Figure S10, Figure S11, Figure S12, Figure S13, Figure S14, Figure S15, Figure S16, Figure S17, Figure S18, Figure S19, Table S1, Table S2, Table S3, and Table S4. This material is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biochem.6b00572.

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Supplementary Figures

Figure S1: Root mean square deviation (RMSD) plot of RS3 mutants in active (top panel) and inactive (bottom panel) states.

Figure S2: Root mean square deviation (RMSD) plot of RS3 mutants in the asymmetric dimer state. Receiver kinase (top panel) and activator kinase (bottom panel) are shown separately.

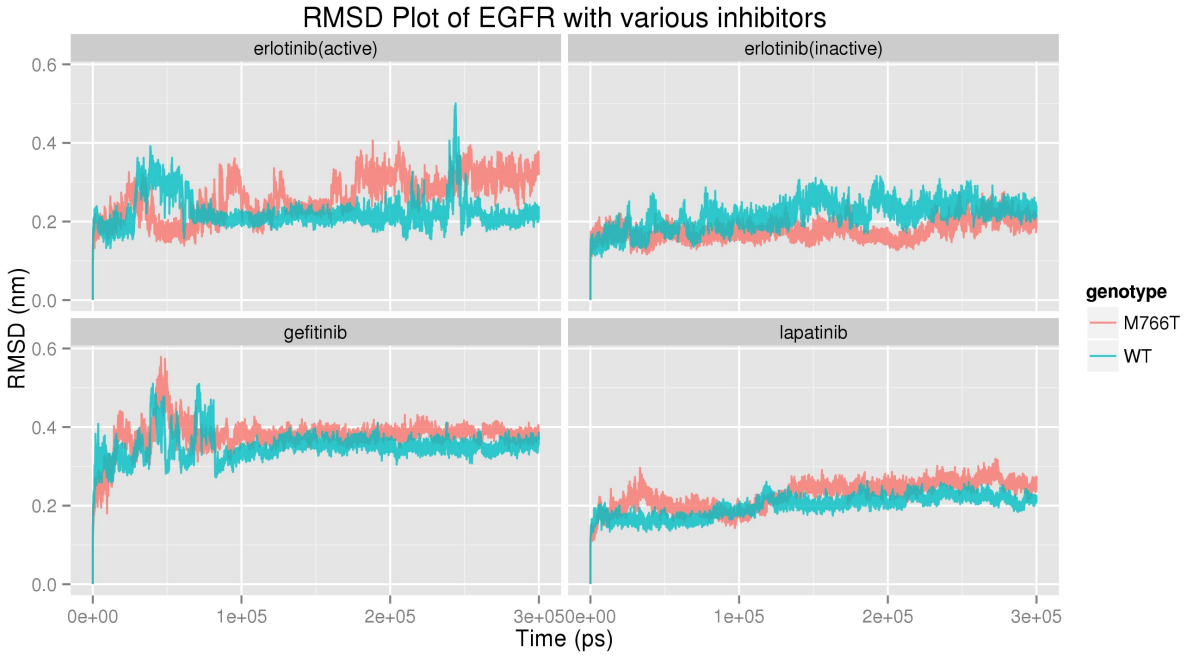

Figure S3: Root mean square deviation (RMSD) plot of WT EGFR and M766T mutant with erlotinib/gefitinib/lapatinib.

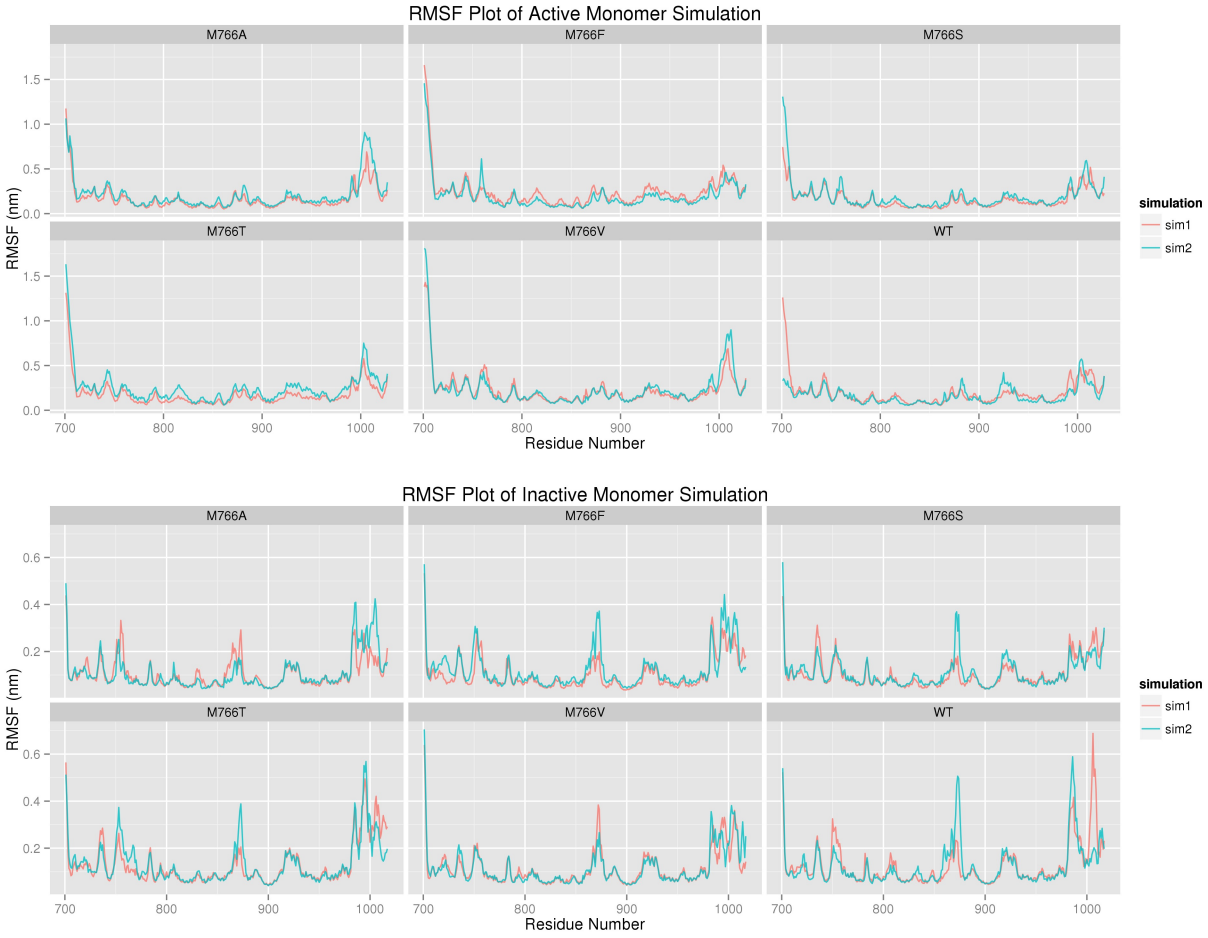

Figure S4: Root mean square fluctuation (RMSF) plot of RS3 mutants in active (top panel) and inactive (bottom panel) states.

Figure S5: Root mean square fluctuation (RMSF) plot of RS3 mutants in the asymmetric dimer state. Receiver kinase (top panel) and activator kinase (bottom panel) are shown separately.

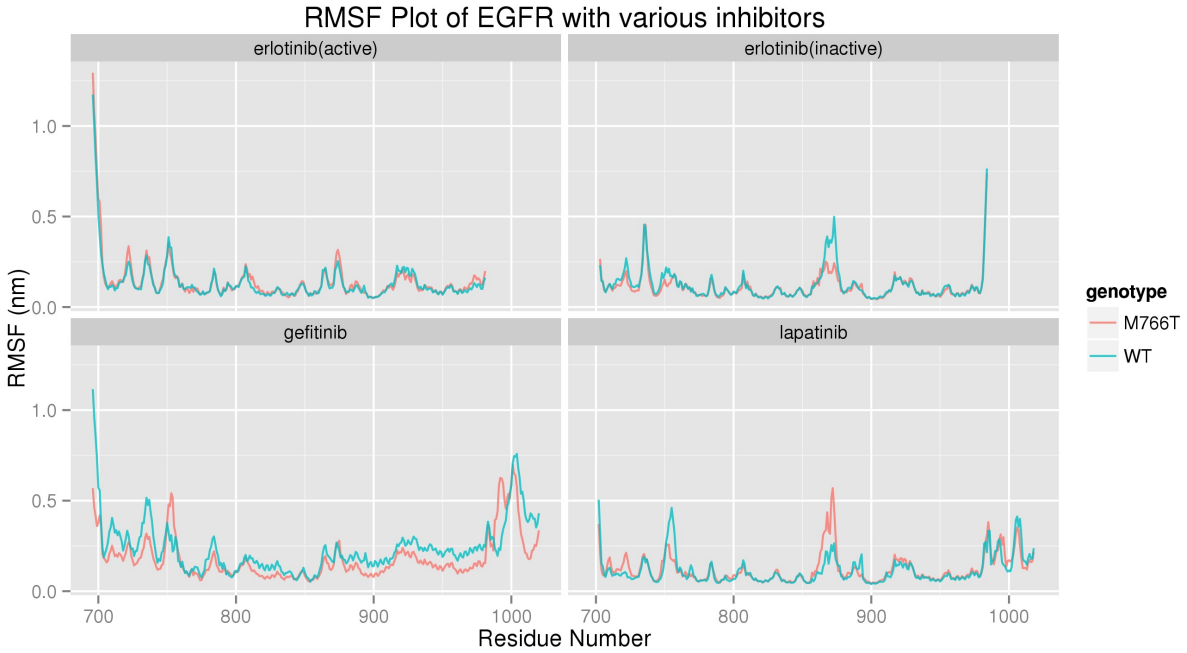

Figure S6: Root mean square fluctuation (RMSF) plot of WT EGFR and M766T mutant with erlotinib/gefitinib/lapatinib.

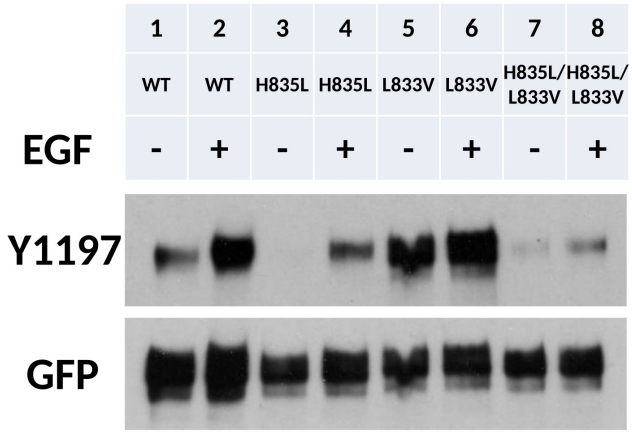

Figure S7: Western blot of H835L (RS1) and co-occurring L833V mutation. Lanes from left to right: WT EGFR (-), WT EGFR (+), H835L (-), H835L (+), L833V (-), L833V (+), H835L/L833V (-), H835L/L833V (+). - and + indicate the absence and presence of EGF stimulation.

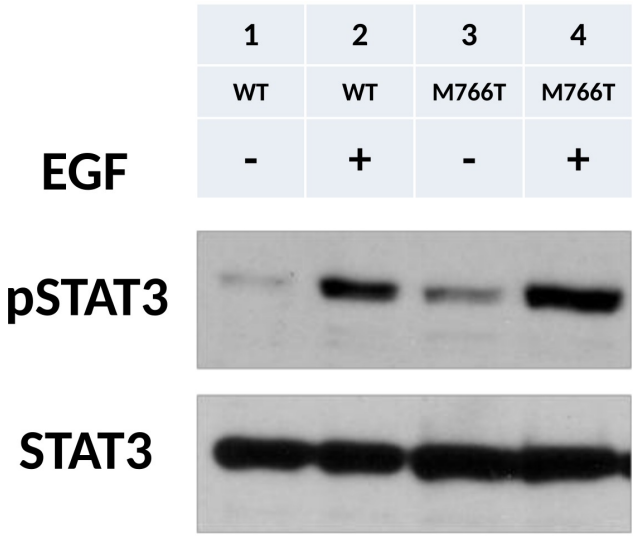

Figure S8: Phosphorylation of STAT3 by M766T EGFR. Lanes from left to right: WT EGFR (-), WT EGFR (+), M766T (-), M766T (+). - and + indicate the absence and presence of EGF stimulation.

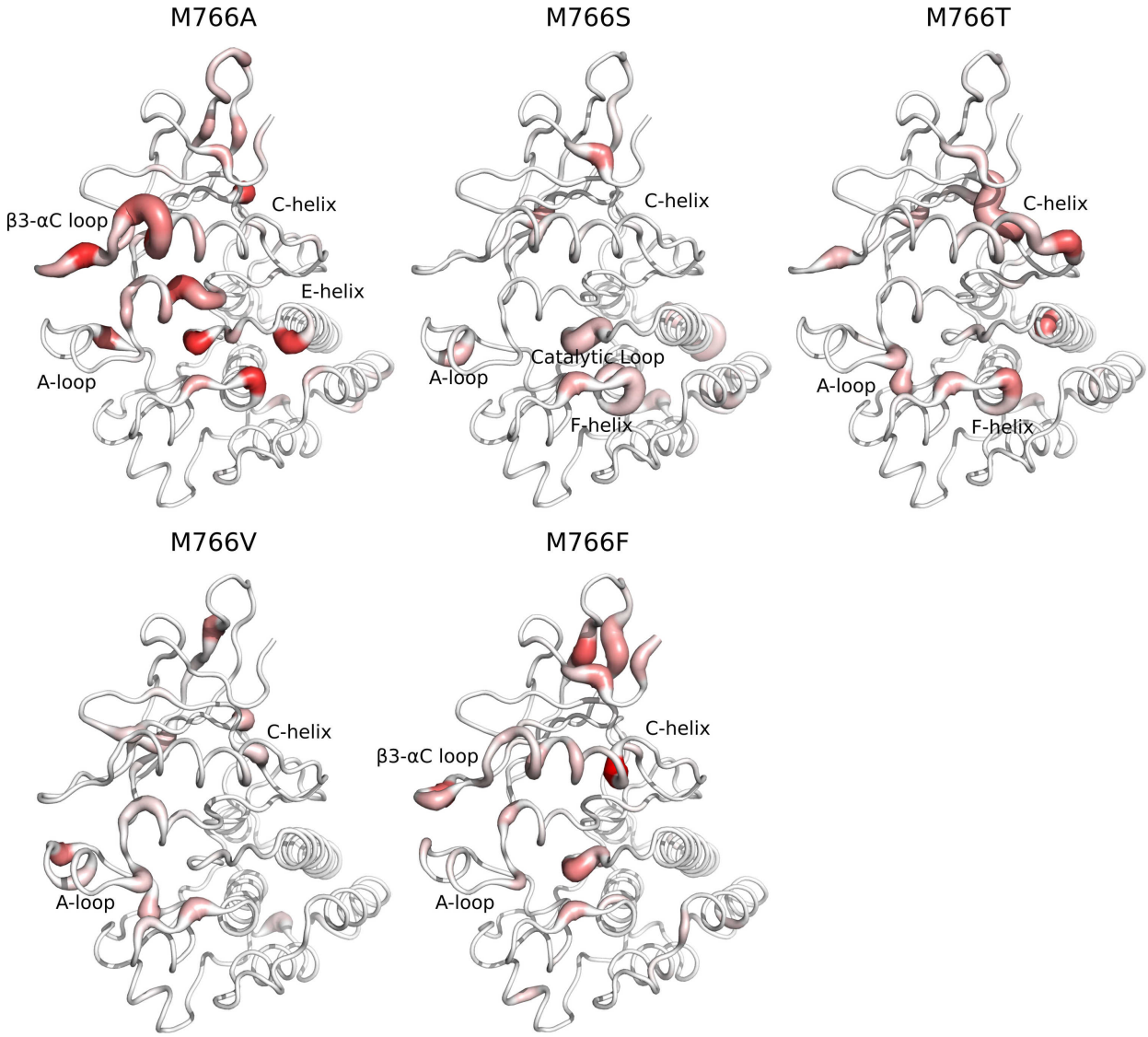

Figure S9: Mapping of Kullback-Leibler (KL) divergent regions in the inactive monomeric state of EGFR.

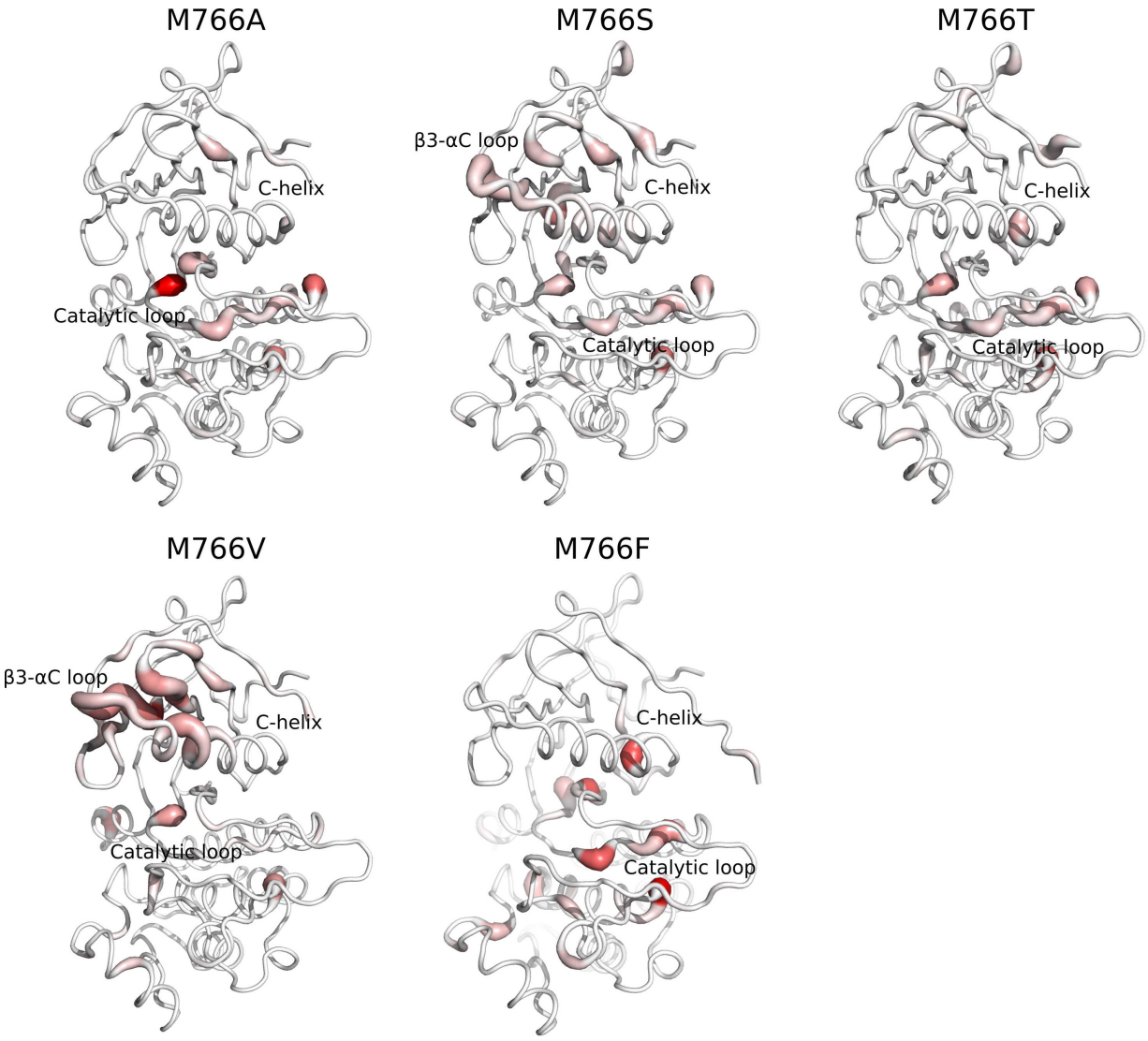

Figure S10: Mapping of Kullback-Leibler (KL) divergent regions in the active monomeric EGFR.

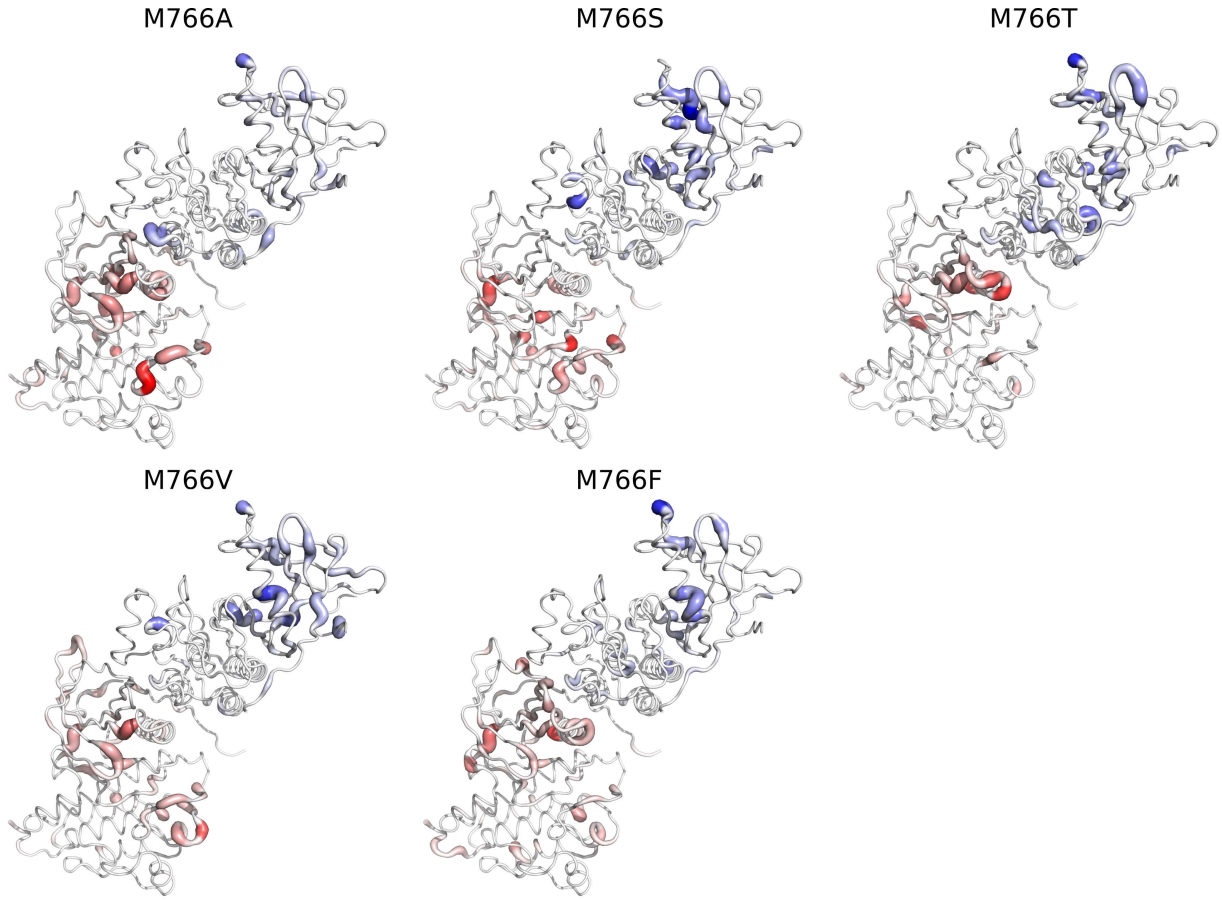

Figure S11: Mapping of Kullback-Leibler (KL) divergent regions in the active asymmetric dimer.

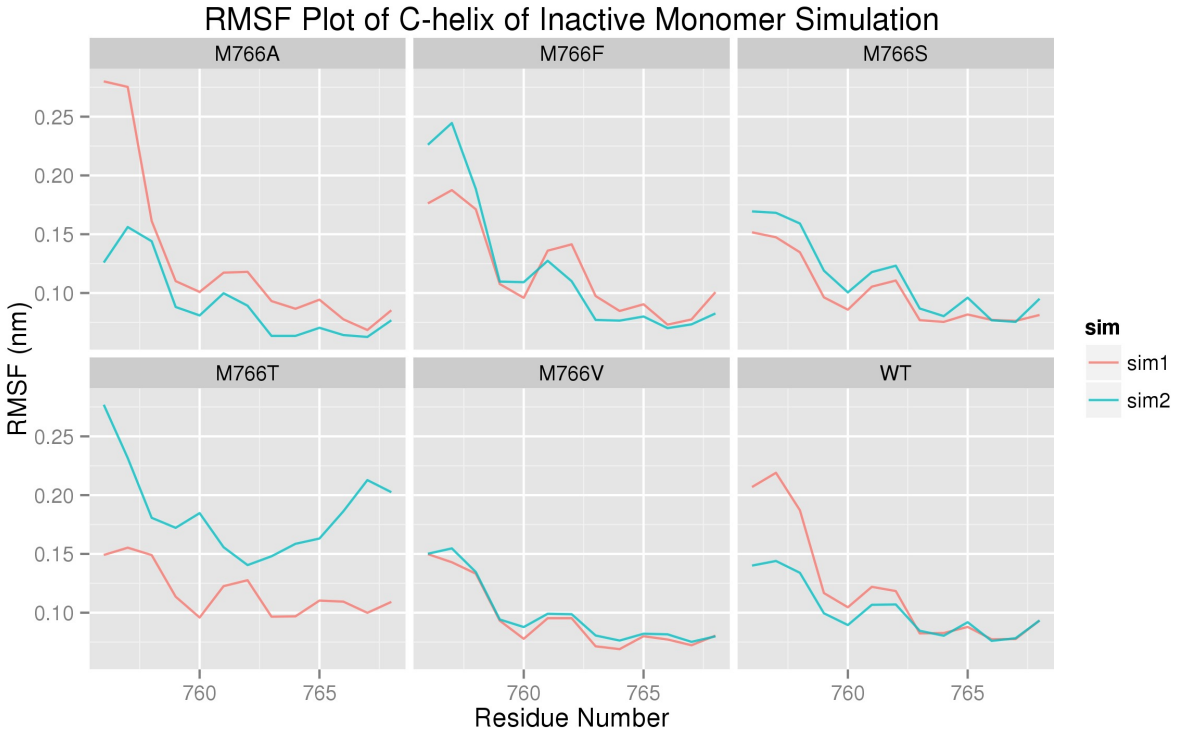

Figure S12: Root mean square fluctuation (RMSF) of the αC-helix in the inactive monomer simulation.

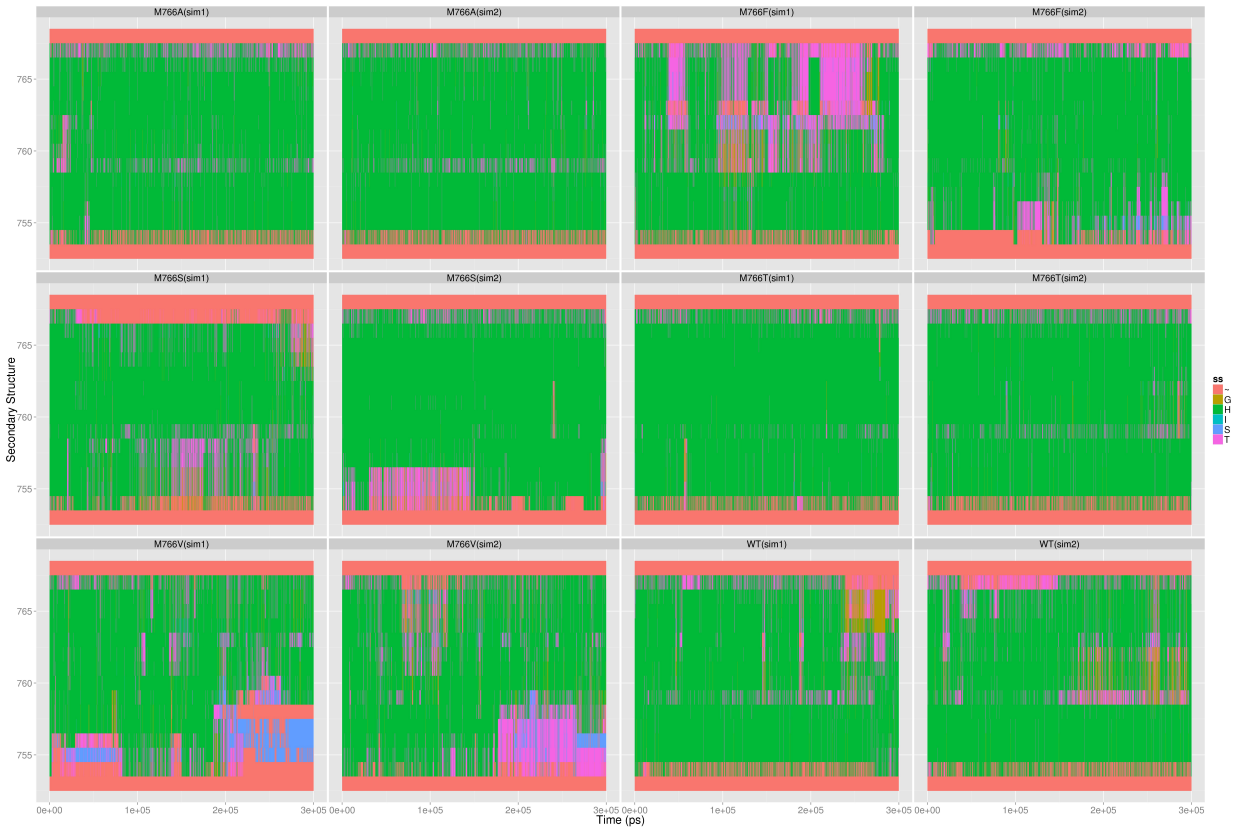

Figure S13: αC-helix secondary structure assignment in the active monomer simulation.

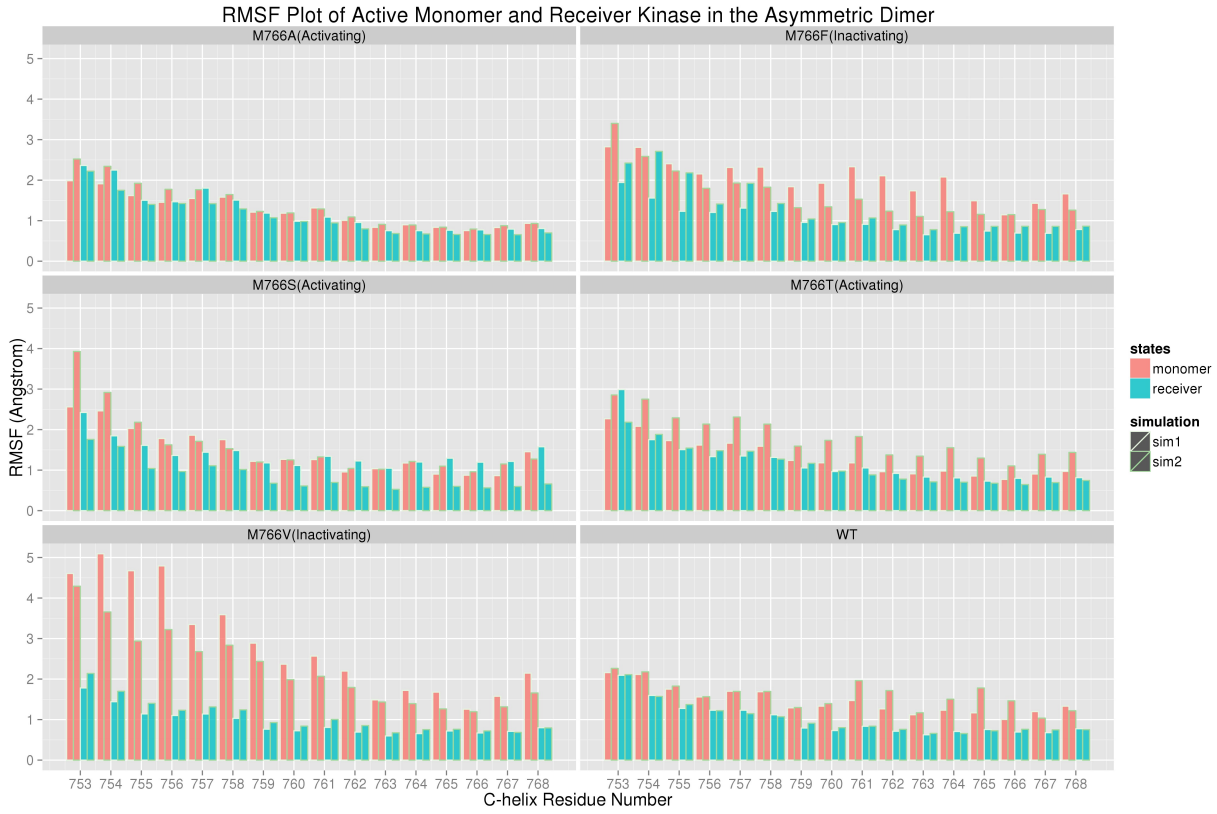

Figure S14: RMSF plot of C-helix residues in the active kinase monomer (red) and receiver kinase (cyan) in the asymmetric dimer

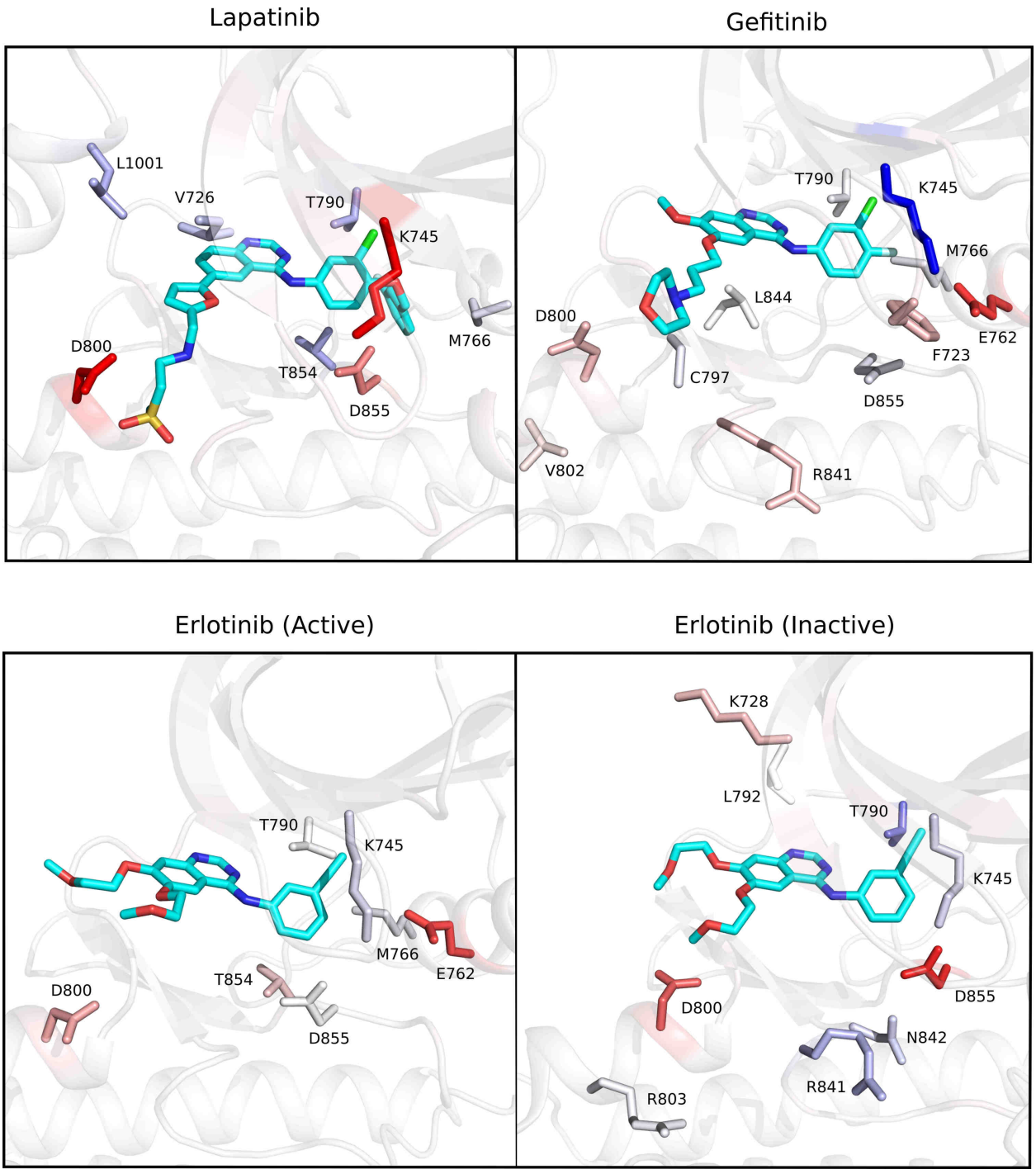

Figure S15: Structural mapping of MM/PBSA energy decomposition analysis. Ligand binding free energy difference between M766T and WT EGFR ($\Delta\Delta G = \Delta G_{M766T} - \Delta G_{WT}$) is mapped to each residue ($\Delta\Delta G < 0$: red; $\Delta\Delta G > 0$: blue). Stick representation is shown if the absolute $\Delta\Delta G$ for the residue is above 2kJ/mol for lapatinib and 1kJ/mol for gefitinib and erlotinib.

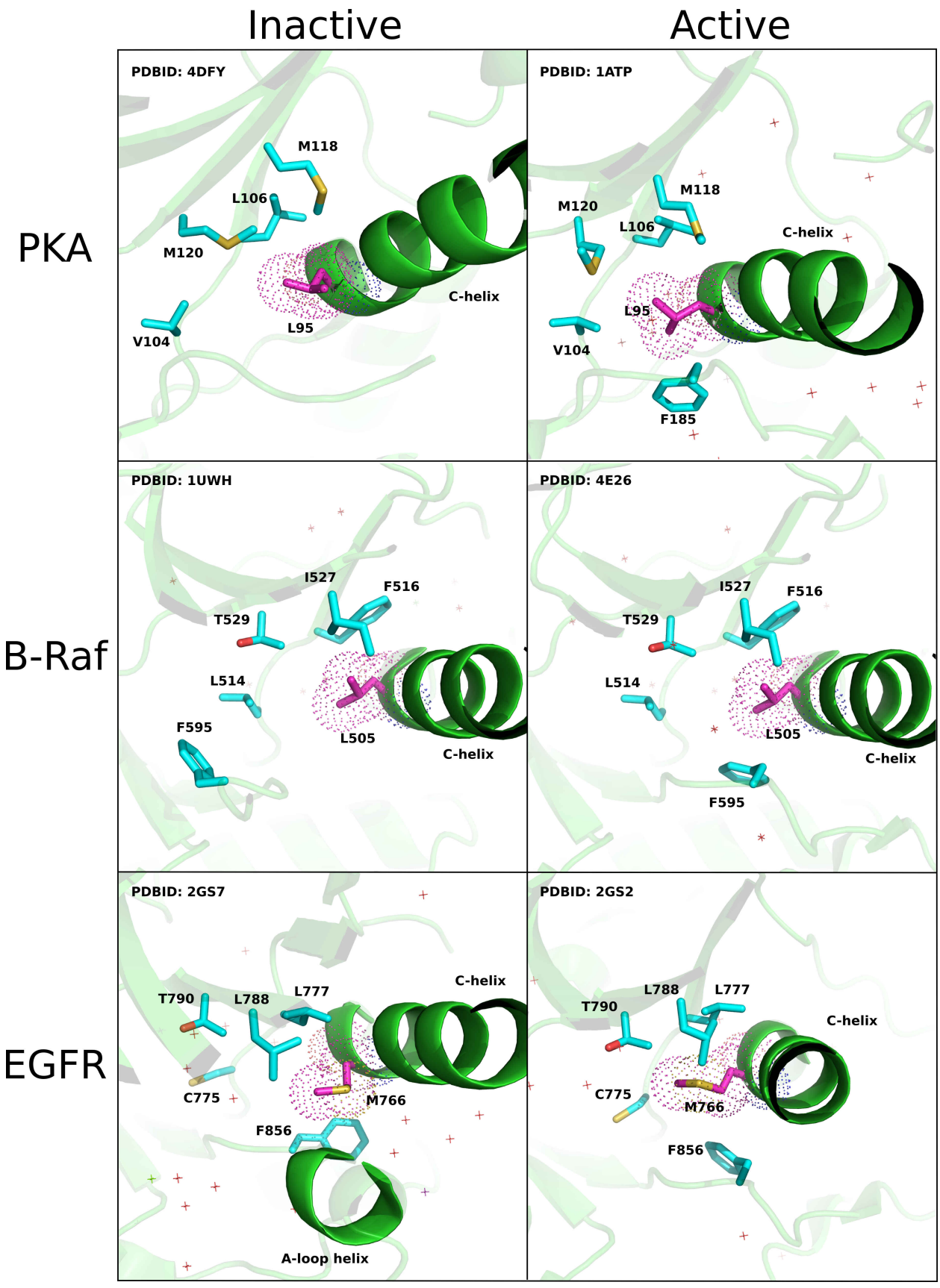

Figure S16: Structural comparison of Protein Kinase A (PKA), B-RAF, and EGFR in active and inactive states.

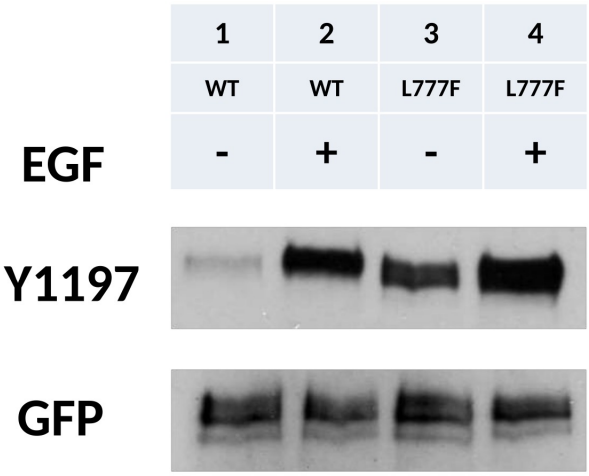

Figure S17: Activity of L777F EGFR. Lanes from left to right: WT EGFR (-), WT EGFR (+), L777F (-), L777F (+). - and + indicate the absence and presence of EGF stimulation.

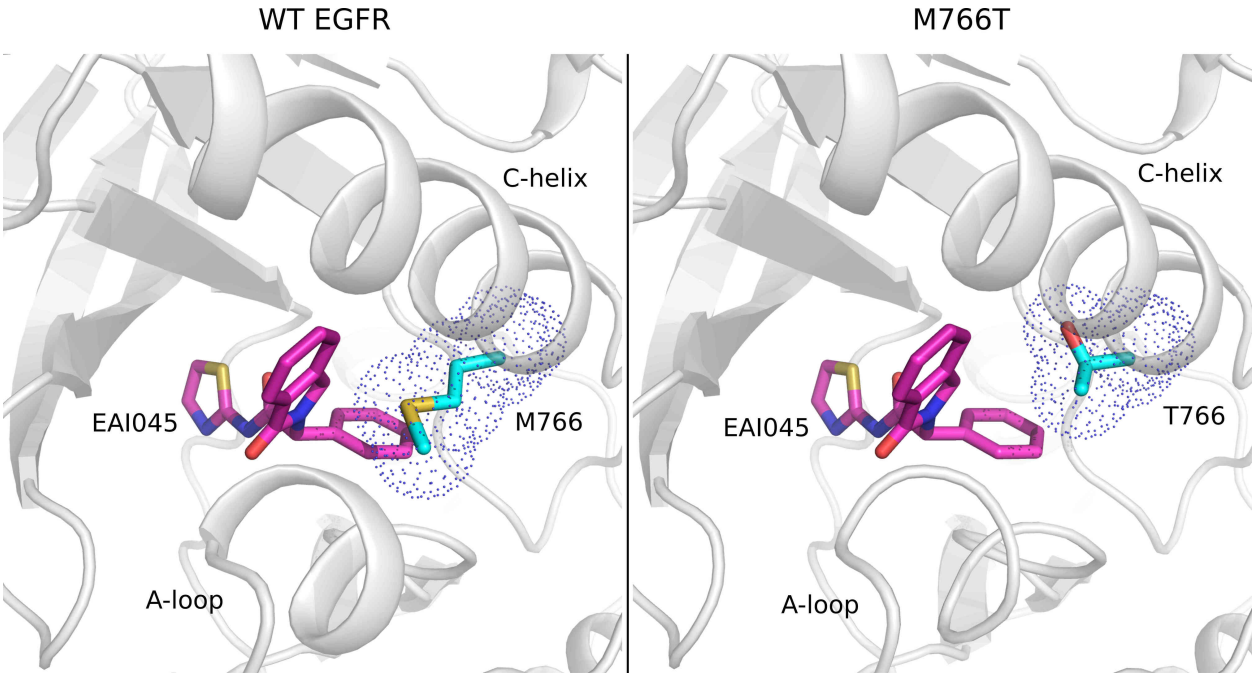

Figure S18: Comparison of the binding mode of WT EGFR and mutant (M766T) to the allosteric inhibitor EAI045.

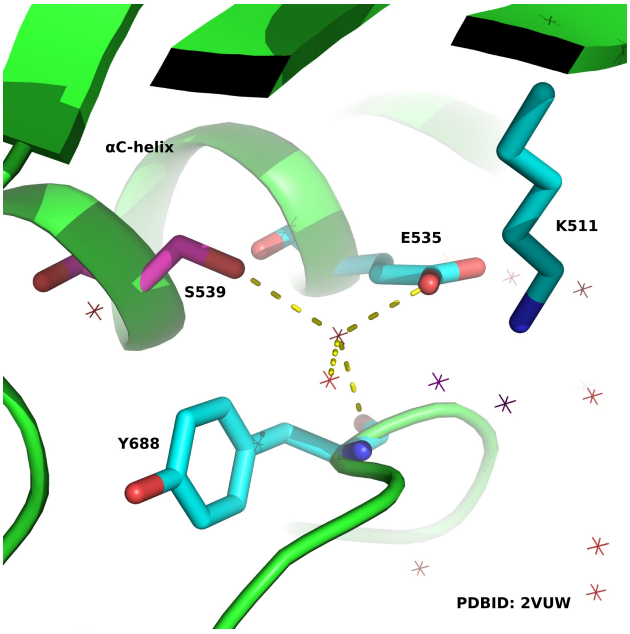

Figure S19: Human haspin kinase conserved a serine at RS3 position and makes water mediated interaction with E535.

Supplementary Tables

Table S1. RS residue mutations and sample ids from cosmic database.

Table S2. Kullback Leibler (KL) divergence score of RS3 mutants in various states (inactive monomer, active monomer and asymmetric dimer)

Table S3. Cancer mutations at the RS3 position in all human kinases. Data Source: COSMIC database.

Table S4. Mutation impact prediction for EGFR RS3 mutants.