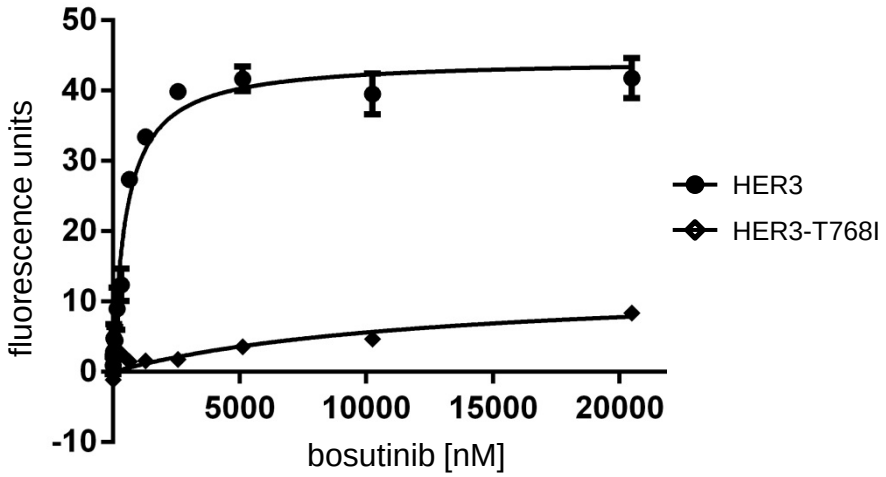

Figure S1, related to Figure 1. Bosutinib binding curves for the wild type HER3 pseudokinase domain and the bosutinib-resistant HER3-T768I mutant. Experiments were performed in solution conditions containing 1.1 mM ATP/Mg^{2+} in order to weaken the apparent HER3/bosutinib Kd to a point accurately measureable in this assay (see Experimental Procedures). Error bars represent SD of fluorescence measurements from duplicate samples.

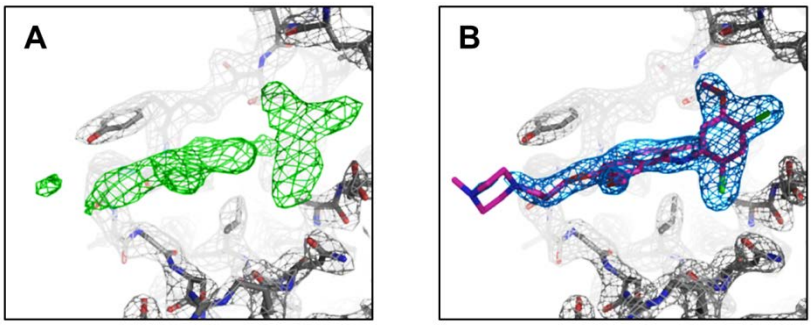

Figure S2, related to Figure 1. Electron density for bosutinib in the HER3/bosutinib crystal structure. (A) Positive electron density (green, Fo-Fc, 2.5σ) observed in the HER3 ATP-binding site after molecular replacement with a ligand-free HER3 kinase domain structure. **(B)** Electron density (blue, 2Fo-Fc, 2.0σ) for bosutinib in the refined structure. Density for HER3 residues (gray, 2Fo-Fc, 2.0σ) is shown in both subfigures.

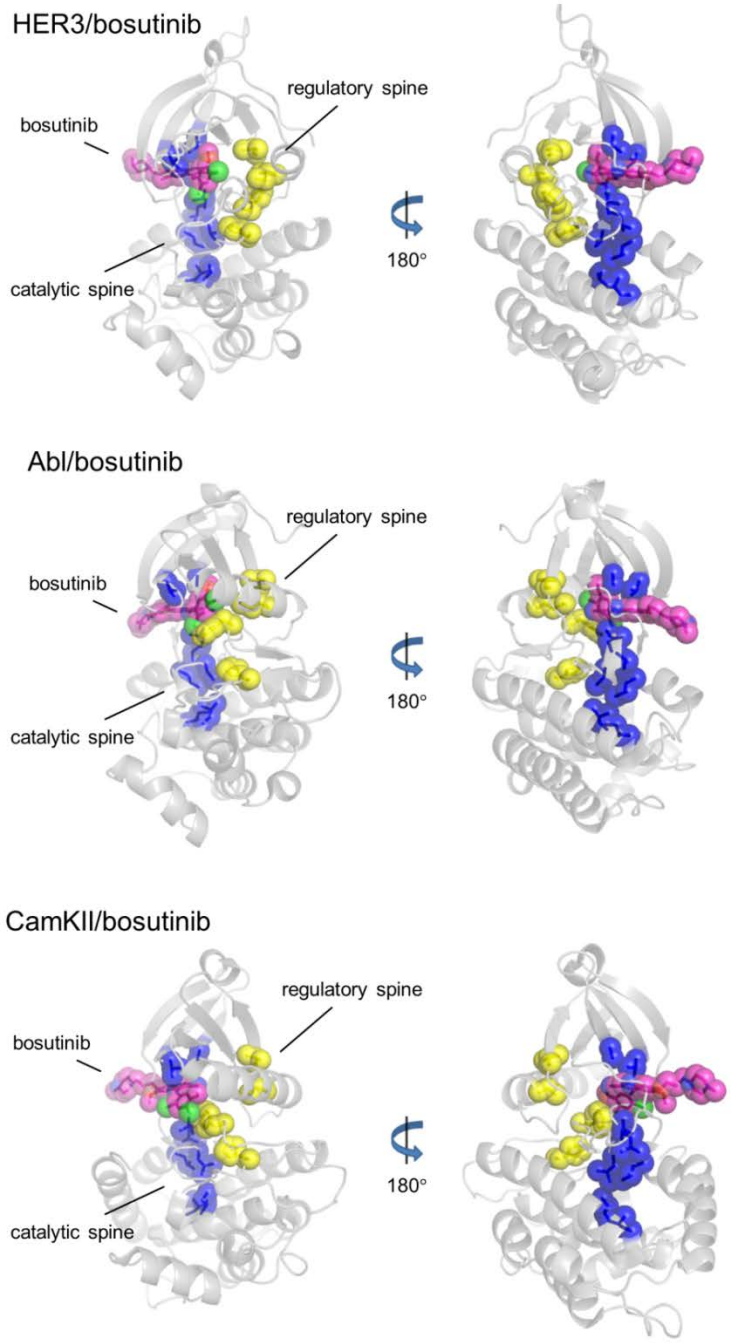

Figure S3, related to Figure 2. Comparison between structures of the bosutinib-bound states of HER3, Abl, and CamKII. Residues within the catalytic spine (blue) and regulatory spine (yellow) are shown in sphere representation for each kinase domain (Taylor and Kornev, 2011). Carbon atoms of bosutinib are shown in magenta. Abl/bosutinib PDB ID: 3UE4. CamKII/bosutinib PDB ID: 3SOA.

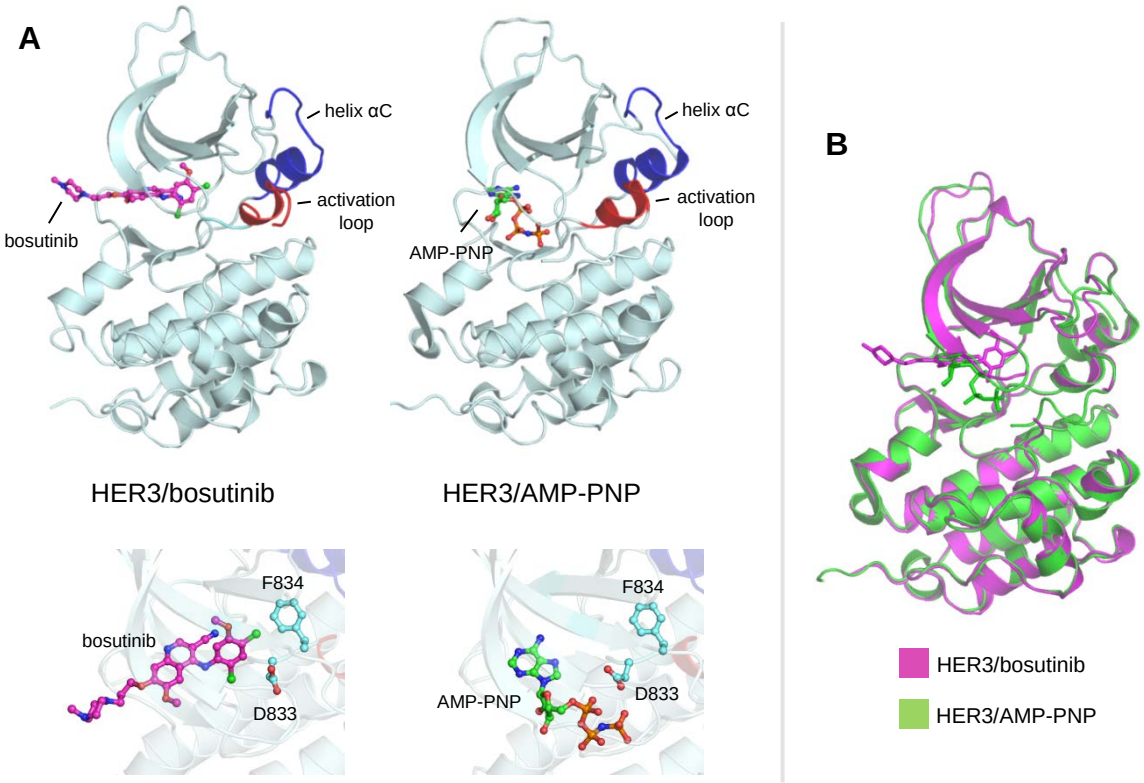

Figure S4, related to Figure 2. Comparison between structures of the HER3 kinase domain bound to bosutinib and AMP-PNP. (A) Cartoon representations highlighting the conformation of helix αC in blue and the activation loop in red (upper panels) and the conformation of the DFG motif (lower panels). **(B)** Overlay of bosutinib- and AMP-PNP-bound HER3 structures showing the high structural similarity of the complexes. HER3/AMP-PNP PDB ID: 3KEX.

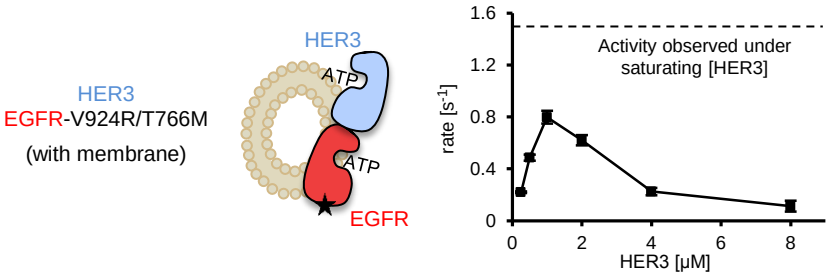

Figure S5, related to Figure 4. EGFR activity measured on the surface of unilamellar vesicles upon titration with the HER3 kinase domain. The observed decrease in kinase activity at higher HER3 concentrations is likely due to the displacement of EGFR from the lipid surface due to limited binding capacity of the membrane. Listed HER3 concentrations reflect the bulk solution concentration. The concentration of the EGFR kinase domain was held constant. Error bars represent SD of two independent measurements.

Supplementary References

Taylor, S.S., and Kornev, A.P. (2011). Protein kinases: evolution of dynamic regulatory proteins. Trends in biochemical sciences ***36***, 65-77.