Supplementary Data and Methods

Trajectory Analysis: Root-mean-square deviation (RMSD)

The long-timescale simulations were analyzed to compute the RMSD of protein backbone relative to the WT+E2 x-ray crystal structure (PDB code: 1GWR), representing the ligand-bound activated conformation of ERα. Using VMD, each simulation frame was aligned to the reference structure by backbone Cα-atoms for the specified chain (A or B) and RMSD measured. The data were smoothed using a Gaussian-weighted running average (σ = 5) and visualized as time series.

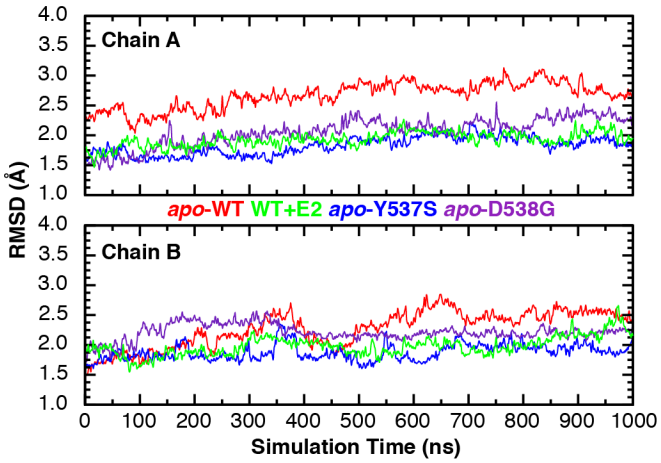

Figure S1. RMSD of receptor backbone relative to ligand-bound ERα. RMSD analysis of the long-timescale MD simulation trajectories quantifies how closely the simulated systems compare to the active ER conformation based on crystallographic data (PDB code: 1GWR). Analysis of chain A demonstrates relative stability of the broader ER protein backbone over the time course of the simulation, in which with the apo-WT system adopts a conformation with an RMSD value consistently higher than all other systems. The unliganded systems bearing the activating mutations, Y537S and D538G, yield RMSD time series that overlap with that of WT-E2. While chain B shows variable values as the system equilibrates over the first half of the simulation, the last 500 ns of the simulation match the data from chain A.