



Figure S5. The ligand specificity of AncSR1 is robust to uncertainty in the reconstruction. A) Distribution of posterior probabilities of 1 million protein sequences assembled by sampling amino acid states at each site from the posterior probability distribution for AncSR1 LBD. The ML reconstruction (AncSR1-ML) and the five alternative sequences with the highest posterior probability (Alt1-Alt5) in the 1 million samples are indicated on the graph. The top table shows the posterior probability (PP) of each sequence in natural logarithmic units, the reduction in posterior probability compared to AncSR1-ML, the PP averaged over sites, and the number of sites with amino acids different from ML-AncSR1. The table below shows the binding pocket residues and their states in the five alternate ancestors. A dot indicates the same state as AncSR1. B) AncSR1-ML and all five alternate ancestors are activated in a dose-responsive manner by estrogens, but C) none are activated by 11-deoxycorticosterone (11-DOC), progesterone, dihydrotestosterone (DHT), or testosterone. The fold activation at 10^{-6} M is shown. The column height and error bars indicate the mean and SEM of three independent experiments, respectively.