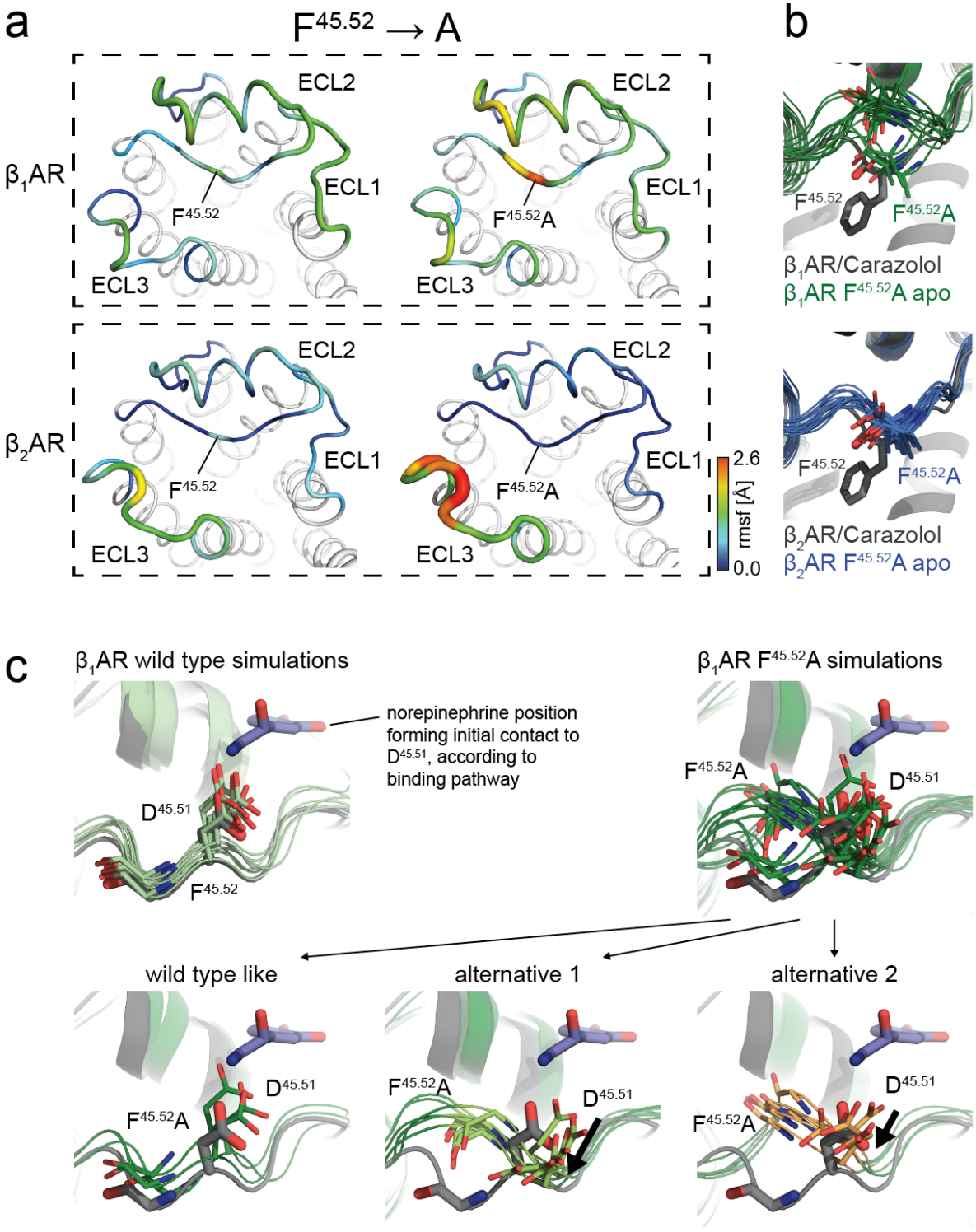

Supplementary information, Fig. S13| The $F^{45.52}A$ mutation alters the conformation of ECL2 of the β_1AR and interferes with the proposed ligand binding pathway.

a, Comparison of the average fluctuation of the extracellular region of the receptors during simulations. The average rmsf values for each residue are mapped onto the energy minimized receptor structures used for the simulations. For β_1AR, the $F^{45.52}A$ mutation introduces an

increased mobility to ECL2 and ECL3, with the greatest effect on position 45.52. For β_2AR, the $F^{45.52}$A mutation only increases mobility of ECL3 while stabilizing the conformation of ECL1 and ECL2. **b**, Only in simulations of β_1AR, $F^{45.52}$A adopts alternative conformations diverging from the respective wild type conformation. The figure displays an overlay of the inactive state crystal structures in complex with carazolol (gray) of β_1AR and β_2AR (PDB ID: 2RH1) with frames of the $F^{45.52}$A mutant simulations of β_1AR (dark green) and β_2AR (blue). The frames were extracted for every 100 ns of the last 500 ns of the simulations. **c**, In simulations of β_1AR, the $F^{45.52}$A mutation interferes with the ligand binding pathway, as it shifts the side chain of $D^{45.51}$ towards the receptor pocket. The frames of the last 500 ns, extracted for every 100 ns, of the β_1AR wild type (light green), the $F^{45.52}$A (dark green) mutant simulations and the inactive state crystal structure in complex with carazolol (gray) are displayed. The backbone atoms of residue 45.52 and the side chain atoms of $D^{45.51}$ are shown as sticks. The displayed structure of norepinephrine is part of the proposed ligand binding pathway and is the position at which the first contact to $D^{45.51}$ is formed. Only one conformation for $D^{45.51}$ and $F^{45.52}$ is observed for the wild type simulations. In contrast, the mutation introduces alternative conformations to the two residues. Three different conformations can be observed for the backbone of $F^{45.52}$A. Here, $D^{45.51}$ either adopts the wild type like conformation or one of two alternative conformations with its side chain shifted towards the receptor pocket. Located deeper in the receptor, the carboxyl group appears less accessible for ligand binding.