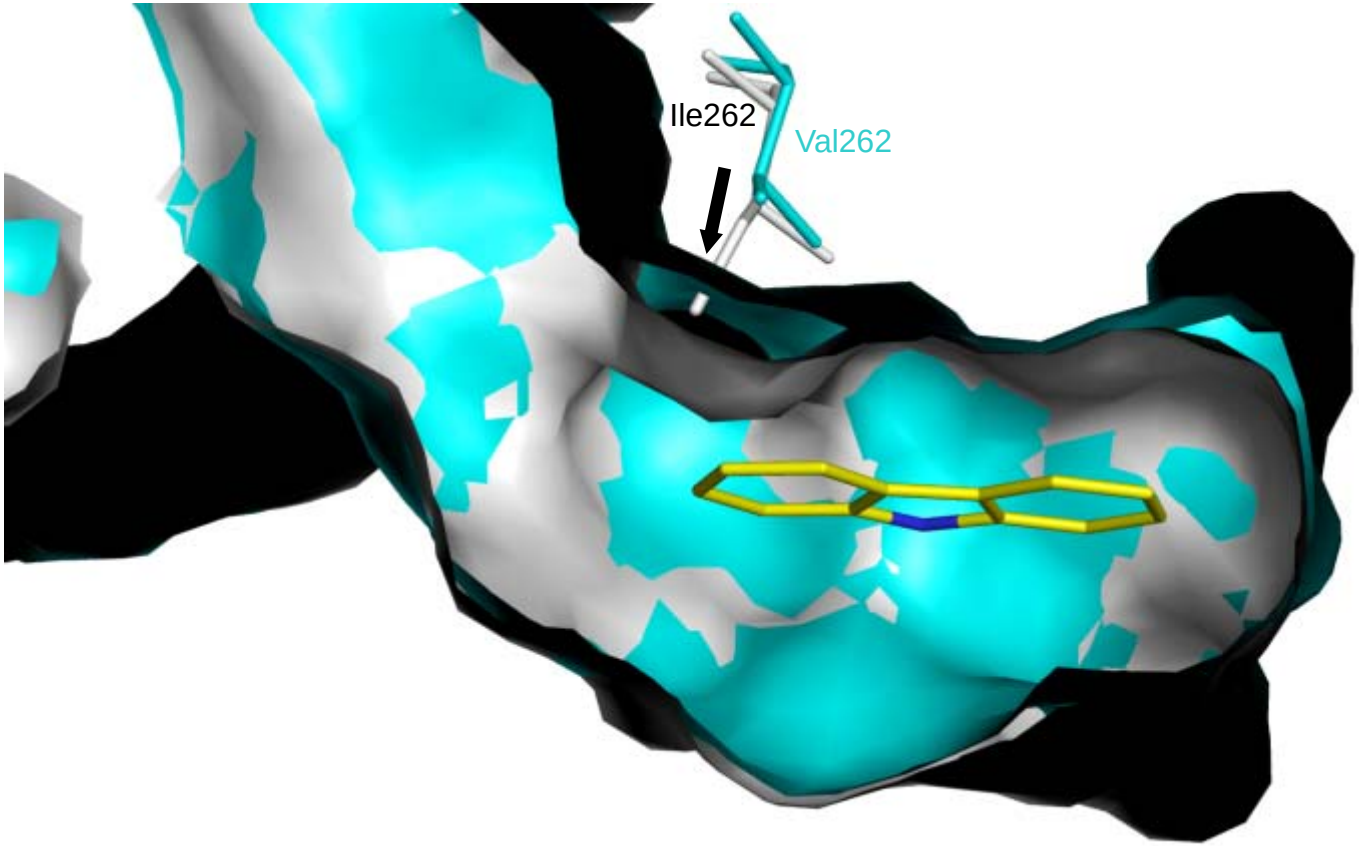

Figure S1. Comparison of the molecular surface of the substrate-binding pocket of wild-type (WT) carbazole 1,9a-dioxygenase (Oxy) and the Oxy derivative I262V in chain B (shown in white and cyan, respectively). Carbazole (CAR) is modeled into the WT Oxy by superimposing the free form of WT Oxy and the WT Oxy complexed with bound CAR. The carbon and nitrogen atoms of CAR are shown in yellow and blue, respectively. The arrow indicates where the molecular surface of the substrate-binding pocket of I262V is expanded. All the structures, WT, WT with CAR, and I262V (PDB ID: 2DE6, 2DE7, and 4NB9, respectively) are superimposed on the Cα of chain B.

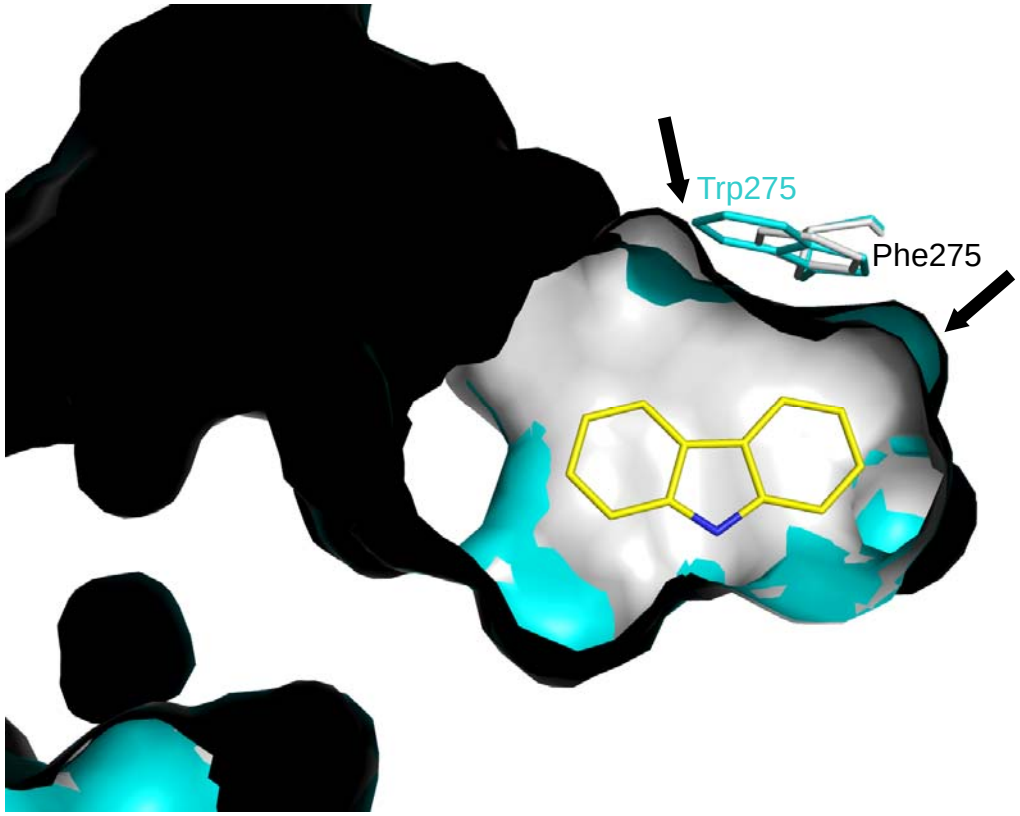

Figure S2. Comparison of the molecular surface of the substrate-binding pocket of wild-type (WT) carbazole 1,9a-dioxygenase (Oxy) and the Oxy derivative F275W in chain B (shown in white and cyan, respectively). Carbazole (CAR) is modeled into the WT Oxy by superimposing the free form of WT Oxy and the WT Oxy complexed with bound CAR. The carbon and nitrogen atoms of CAR are shown in yellow and blue, respectively. Arrows indicate where the surface shape of the substrate-binding pocket has changed. All the structures, WT, WT with CAR, and F275W (PDB ID: 2DE5, 2DE7, and 4NBC, respectively) are superimposed on the Cα of chain B.

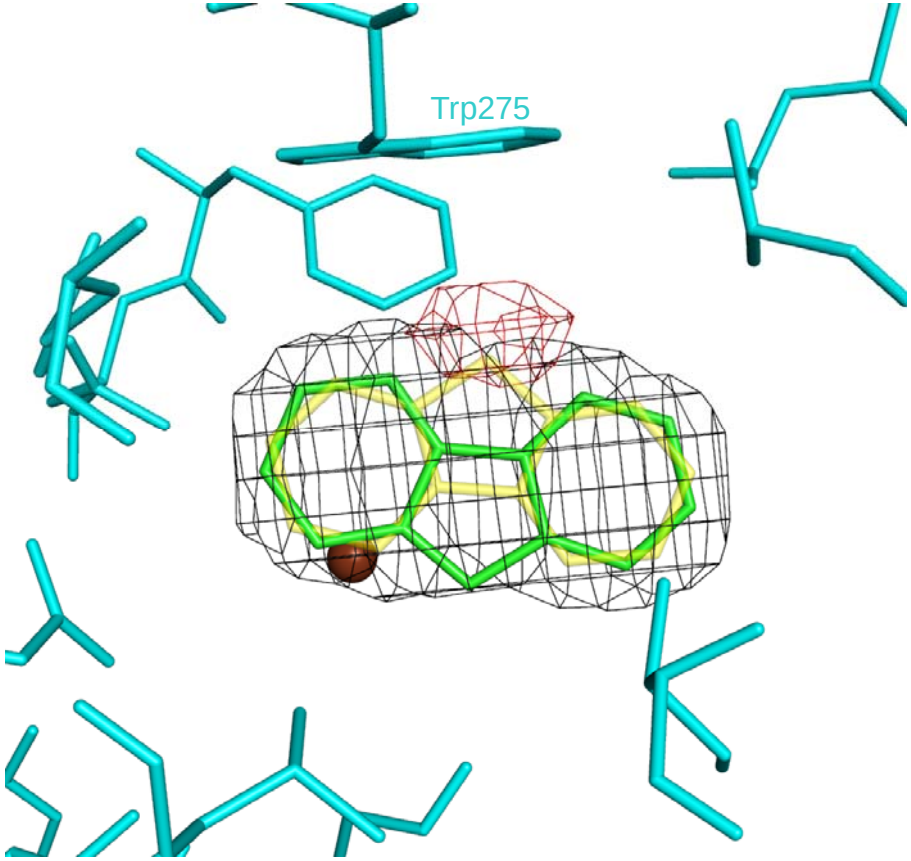

Figure S3. Electron density map of the substrate-binding pocket in chain C of the carbazole 1,9a-dioxygenase (Oxy) derivative F275W with bound fluorene (FN) (1.0 σ 2Fo-Fc: black mesh) and omit map (2.5 σ Fo-Fc: red mesh) of F275W with the FN flipped 180° around its long axis (green sticks) from its original position, in which FN was fit to the electron density map (yellow sticks). The electron density omit map appeared when C9 of FN was placed with the same orientation as carbazole (CAR) in wild-type Oxy complexed with CAR (red mesh), whereas the omit map disappeared when the FN was flipped.

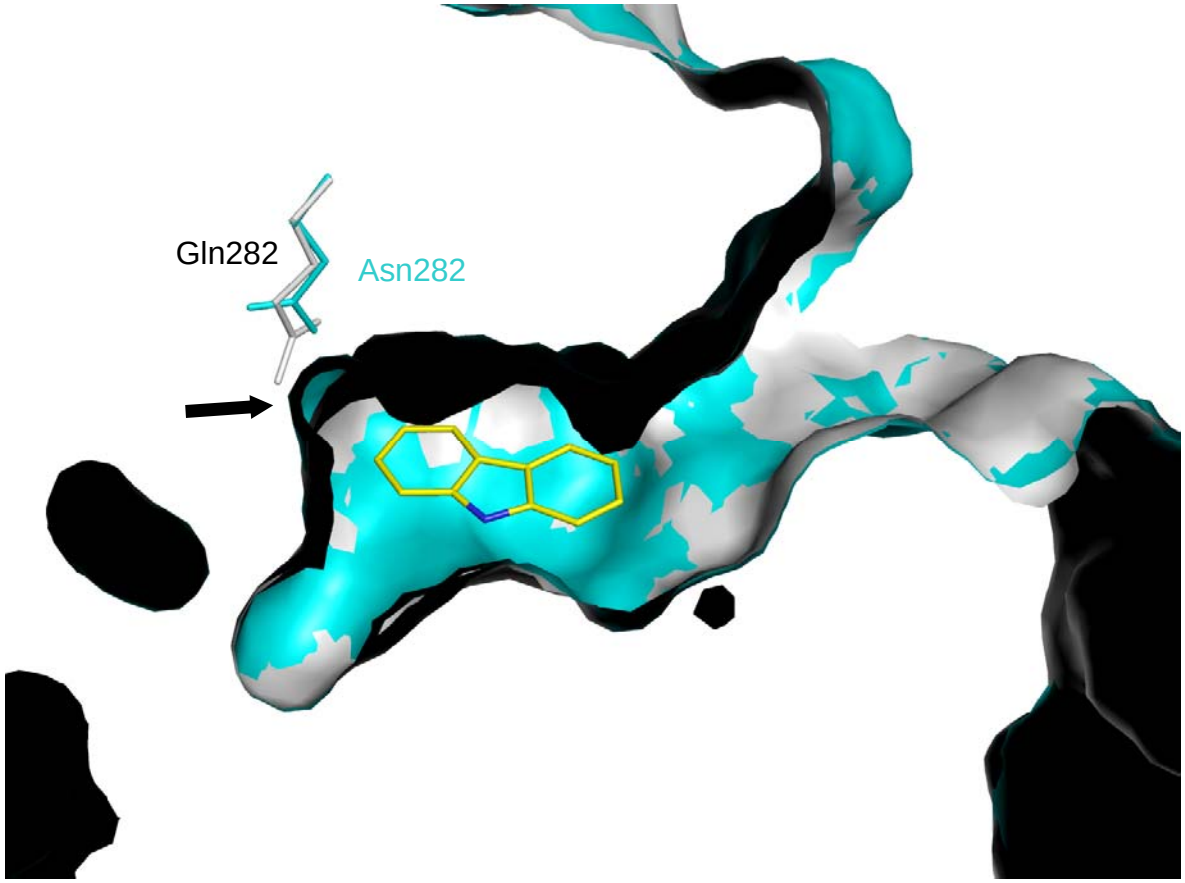

Figure S4. Comparison of the molecular surface of the substrate-binding pocket of wild-type (WT) carbazole 1,9a-dioxygenase (Oxy) and the Oxy derivative Q282N in chain B (shown in white and cyan, respectively). Carbazole (CAR) is modeled into the WT Oxy by superimposing the free form of WT Oxy and the WT Oxy complexed with bound CAR. The carbon and nitrogen atoms of CAR are shown in yellow and blue, respectively. An arrow indicates where the substrate-binding pocket surface is depressed. All the structures, WT, WT with CAR, and Q282N (PDB ID: 2DE5, 2DE7, and 4NBF, respectively) are superimposed on the Cα of chain B.

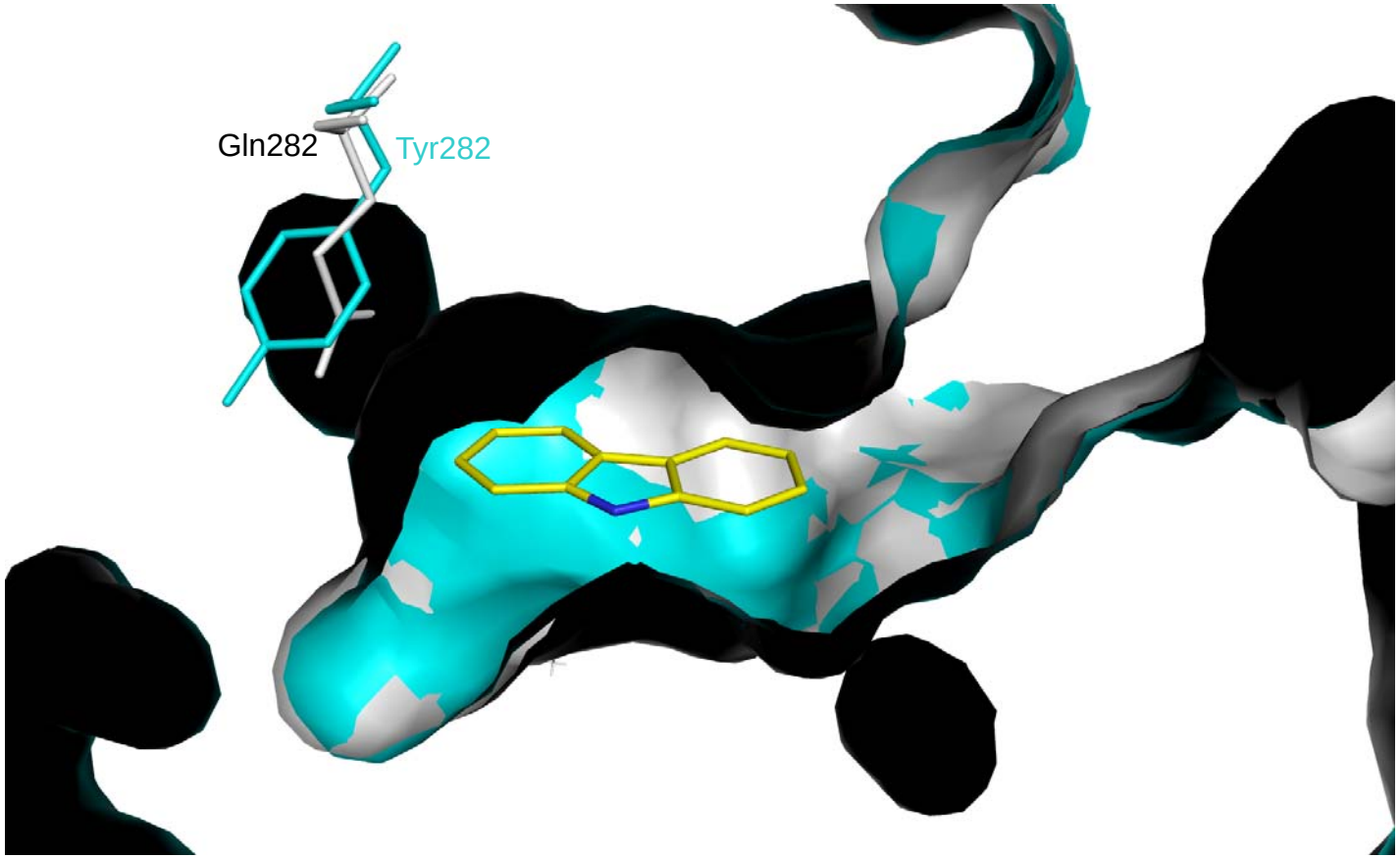

Figure S5. Comparison of the molecular surface of the substrate-binding pocket of wild-type (WT) carbazole 1,9a-dioxygenase (Oxy) and the Oxy derivative Q282Y in chain B (shown in white and cyan, respectively). Carbazole (CAR) is modeled into the WT Oxy by superimposing the free form of WT Oxy and the WT Oxy complexed with bound CAR. The carbon and nitrogen atoms of CAR are shown in yellow and blue, respectively. There is no significant difference between the WT Oxy and substrate-free form of Q282Y. All the structures, WT, WT with CAR, and Q282Y (PDB ID: 2DE5, 2DE7, and 4NBG, respectively)are superimposed on the Cα of chain B.

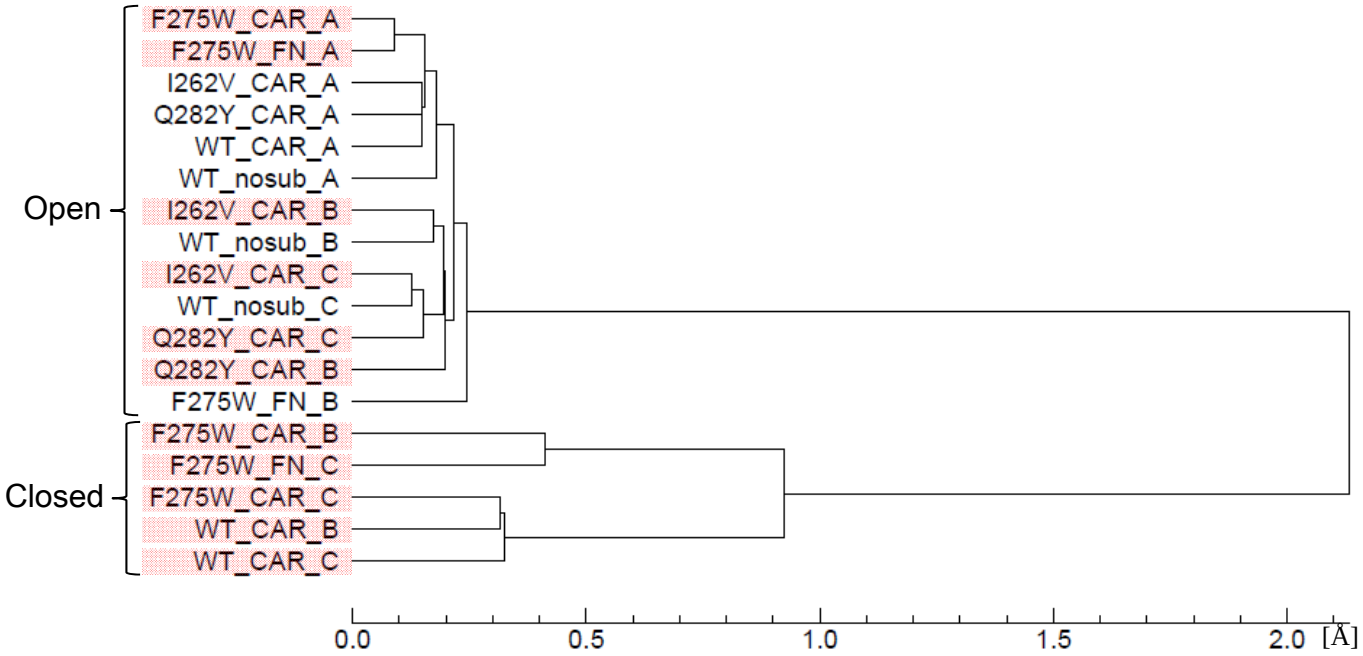

Figure S6. Hierarchical clustering analysis based on the pairwise root-mean-square-deviation (RMSD) distance matrix using each chain in the determined crystal structures with and without substrate. The structures that contain substrates are shown with a pink background. The chains in which the entrance to the substrate-binding pocket is closed (amino acid residues Leu202-Thr214 and Asp229-Val238 are shifted upon substrate binding) or open are also indicated.