Supplementary Material for

Structural And Functional Characterization Of An Anesthetic Binding Site In The Second Cysteine-Rich Domain Of Protein Kinase Cδ

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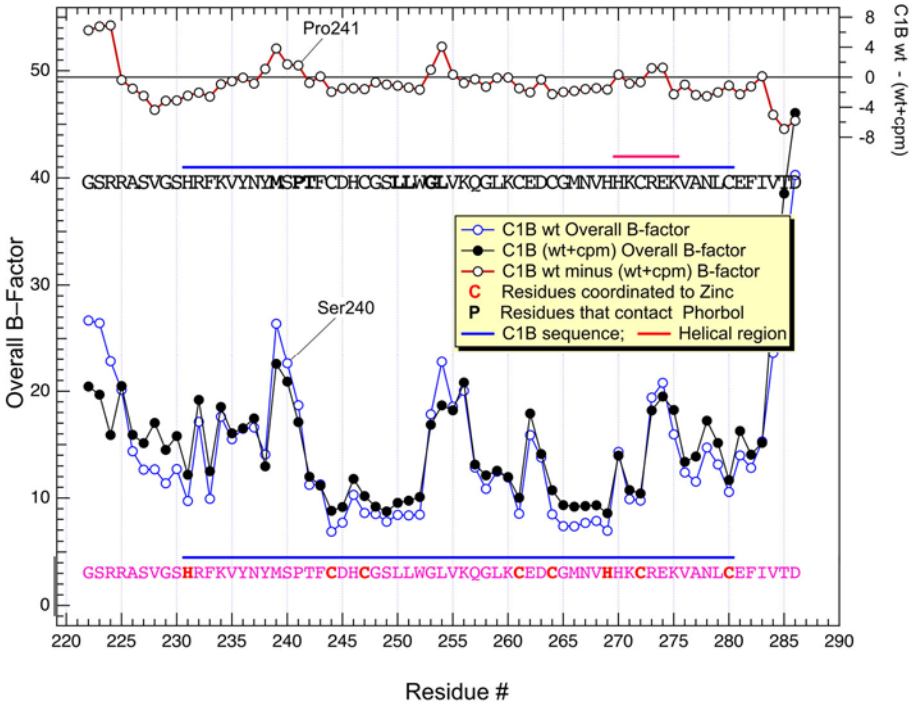

FIGURE S1. **Overall B-factors for molecule B of PKCδ C1B with and without cyclopropylmethanol (cpm) bound.** B-factors (left axis, Å^2) are plotted against residue number, and their difference is plotted in the upper part of the diagram (right axis). The sequence is indicated, including the vector-derived residues. A blue superscript line indicates the 50 residues of C1B. Residues coordinated to zinc are labeled in red in the lower sequence, whereas those previously determined to be in contact with bound phorbol ester (1) are in bold in the upper sequence. The red superscript line indicates the only helical region.

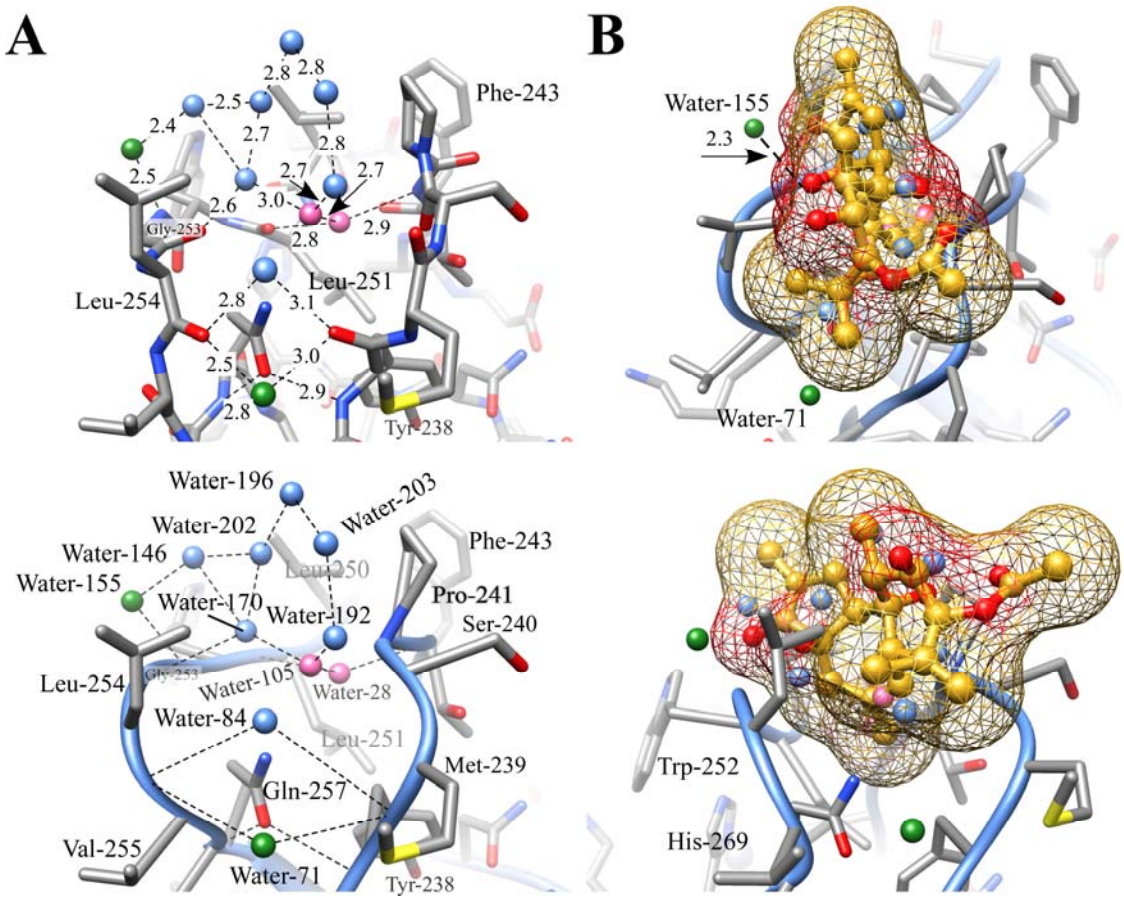

FIGURE S2. **The role of water molecules in the phorbol–binding cleft.** Upper and lower panels are top and side views respectively. (***A***) There are eleven water molecules in the phorbol binding cleft that form hydrogen bonds between themselves and the backbone of the phorbol binding loops. (***B***) Bound phorbol acetate (gold carbons) from the Hurley structure (1) is superimposed on our structure. Water-155 and Water-71 (green) remain in van der Waals contact with the phorbol. The nine other waters (blue and orchid) are displaced. The orchid waters are centered on phorbol atoms or bonds, for example, Water-28 occupies the same space as oxygen-20 of the phorbol ester. They are hydrogen-bonded to Phe-243's backbone amide (2.9 Å) and to Leu-251's backbone carbonyl (2.8 Å). Other phorbol oxygens are solvent exposed and form hydrogen bonds to Gly-253's amide (O3, 3.0 Å; O4 2.64 Å) and Met-239's carboxyl (OA1, 3.1 Å).

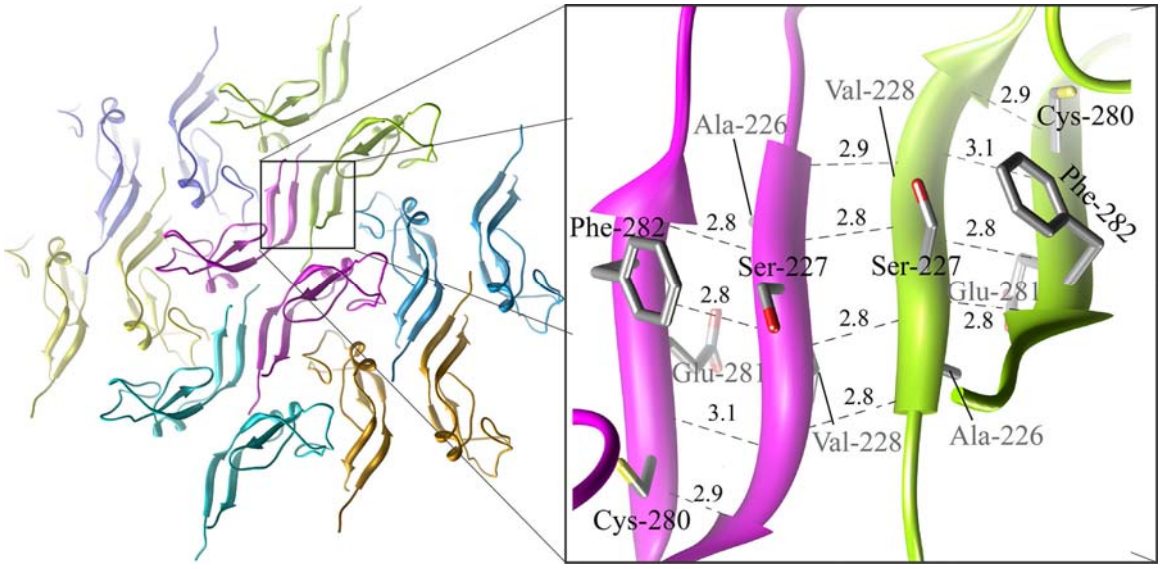

FIGURE S3. T**he role of the construct's residues in the crystal.** On the left, the arrangement of C1B in the crystal is shown with pairs of molecules in the unit cell colored identically. On the right, a detailed view shows how two two–stranded anti–parallel intramolecular β-sheets interact to form a four–stranded anti–parallel intermolecular β-sheet between symmetry–related molecules. Hydrogen bonds are shown as dashed lines and distances are in Ångstrom units. The figure was prepared with Chimera (2).

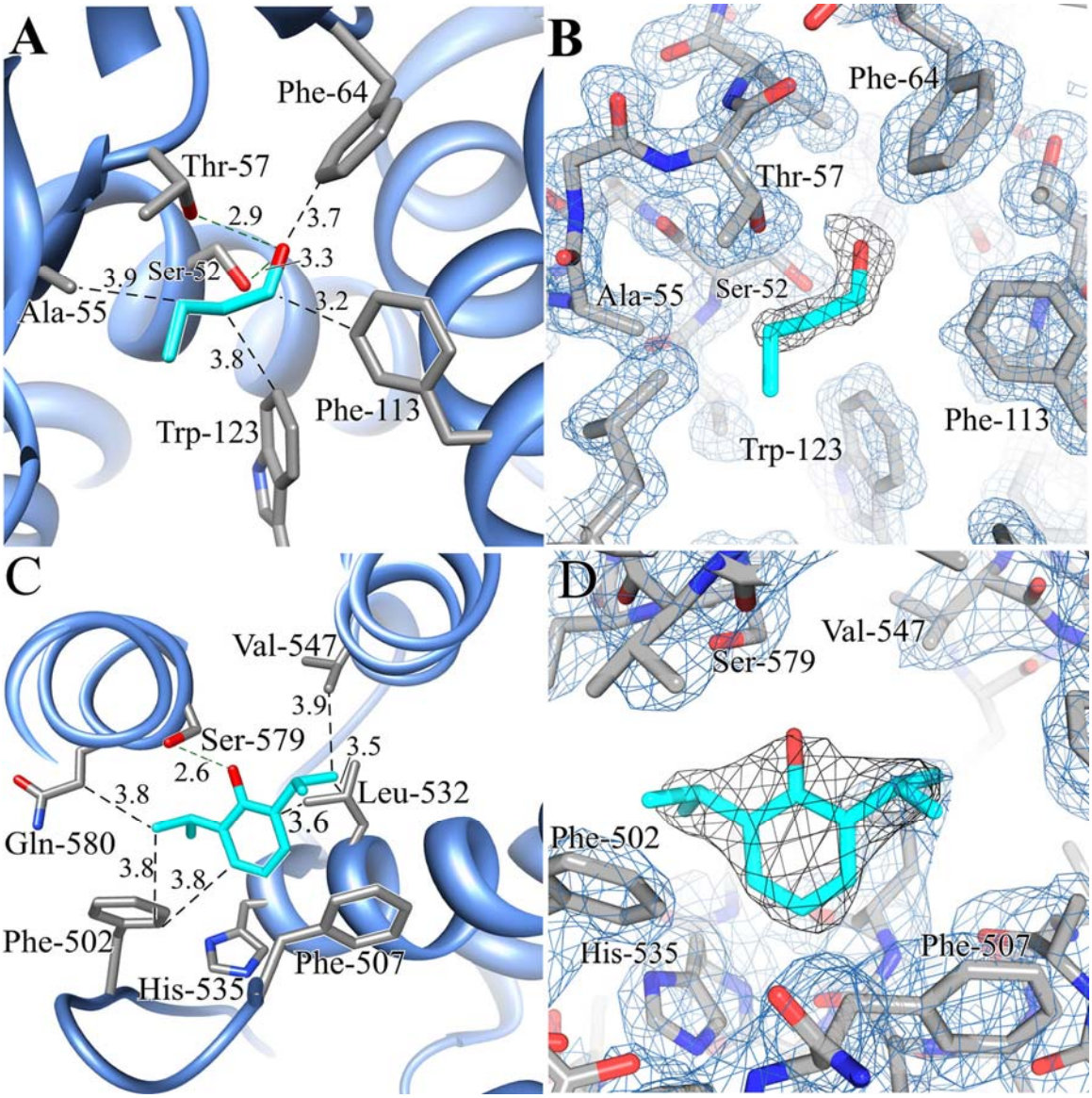

FIGURE S4. **For comparison two published structures of other alcohol and anesthetic binding sites are shown.** (***A***) Butanol (cyan carbons) binding to LUSH (corn blue ribbons; (3)); PDB code: 1OOH; 1.25 Å resolution) is stabilized by two hydrogen bonds, to Ser-52 and Thr-57, green dashed lines, and by van der Waals contacts with neighboring residues (Ala-55, Phe-64, Phe-113 and Trp-123) in a buried, well defined hydrophobic pocket bounded by two helices and the C-terminal strand. Dashed lines indicate contacts within 4 Å. (***B***) The same angle of view as A, showing the electron density at 1σ around butanol (dark grey mesh) and the neighboring residues (sky blue mesh). The lack of density around butanol's terminal methyl group suggests

that it has no preferred conformation. The structure factor file was kindly provided by Dr. David Jones. (***C***) Propofol (cyan carbons) binding to HAS (4); PDB code; 1e7a; 2.2 Å resolution) is stabilized by a hydrogen bond to Ser-579 and by van der Waals contacts; those within 4.0 Å are indicated by dashed lines. The pocket is formed by three helices. (***D***) The same angle of view as C, showing the electron density at 1σ around propofol (dark grey mesh) and the neighboring residues (sky blue mesh).

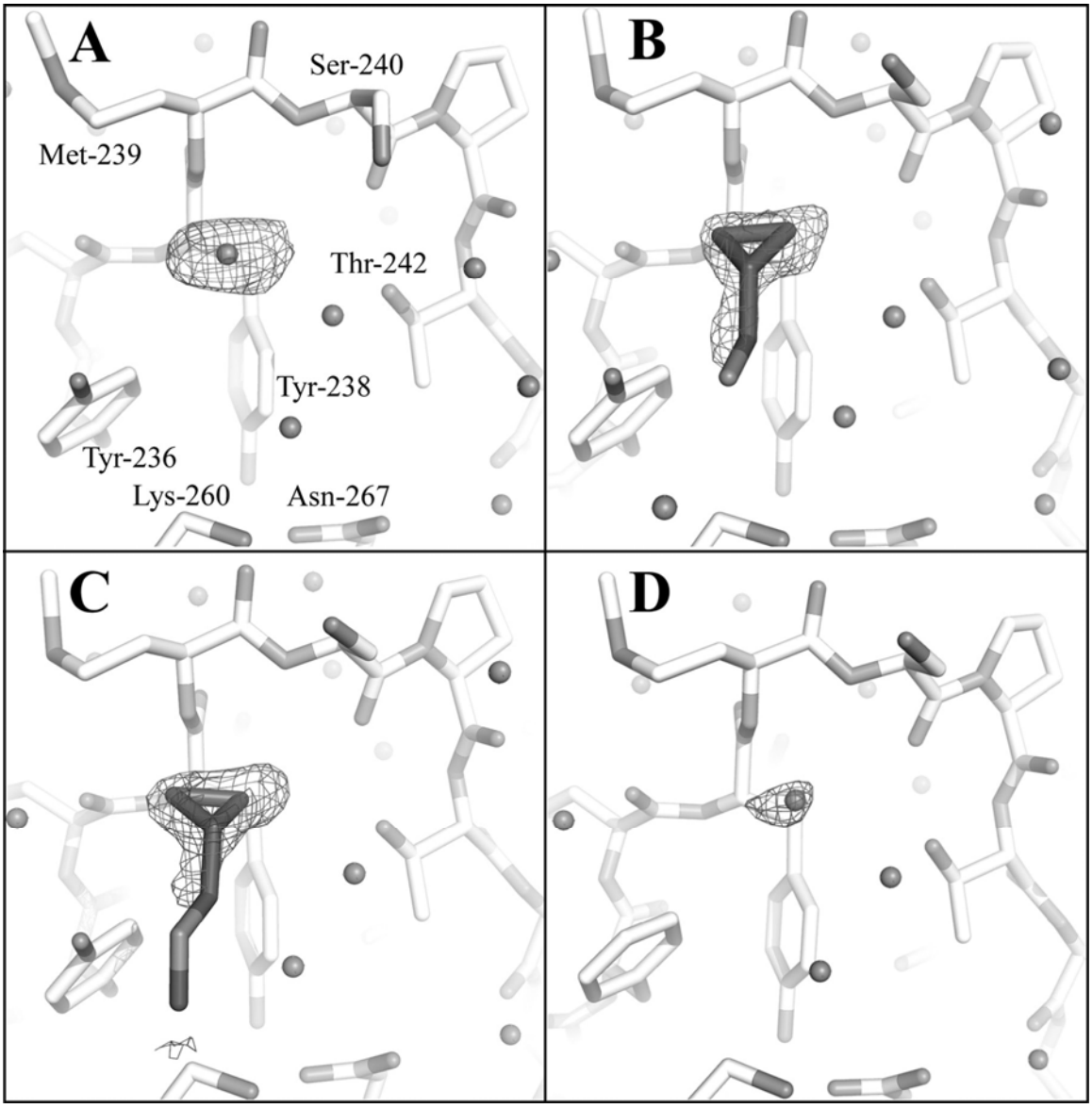

FIGURE S5. **Omit maps confirm ligand assignments.** Omit maps are shown in mesh at 2.5σ. The panels are arranged and labeled as in Fig. 2. Wild type (A), with CPM (B) or CPE (C) and Tyr-236-Phe (C). See text for discussion. The figures were prepared using Pymol (5).

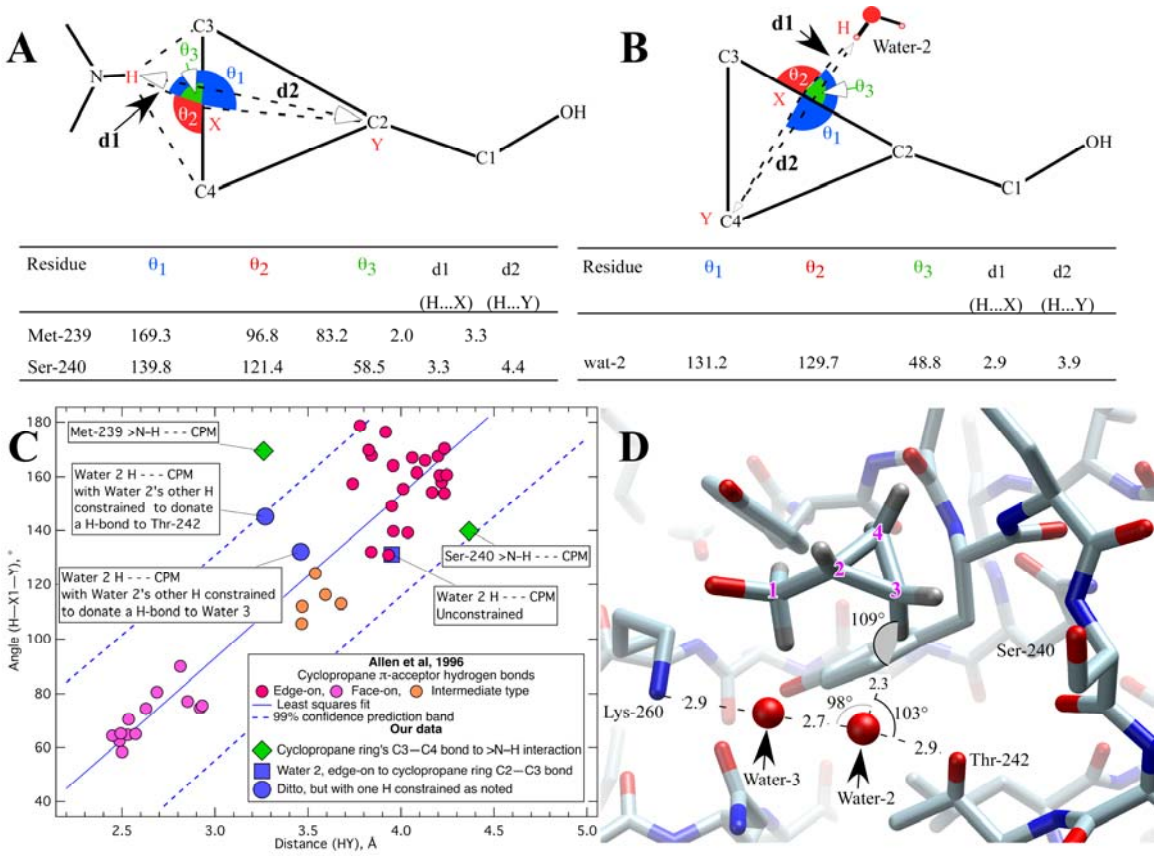

FIGURE S6. **Characterization of potential hydrogen bonds between the cyclopropane ring and PKCδ C1B.** ***(A & B)***. Following the analysis of Allen et al. and using their terminology (42), we characterized the edge–on J–H …π interactions of the cyclopropane ring with potential neighboring backbone amides (**A**: J = Met-239 or Ser-240) and solvent H–donors (**B**: J = Water-2). Hydrogens were added at the theoretically calculated positions using the program Chimera (59). ***(C)***. The data from Allen et al.'s survey of small molecules (colored circles, see key) is plotted and compared to the results of our analysis. The backbone amides of Met-239 and Ser-240 (green diamonds) are in the region for edge–on hydrogen bonding and close to the 99% prediction band (blue dashed lines) derived from a database of small molecules. ***(D)*** When Water-2's O–H position is optimized as an O–H … π ring donor *(*see *B)*, it has excellent geometry and it can also accept a hydrogen bond from ring C3–H (blue square in *C*; geometry in *D*). In addition, when we constrain Water-2's other hydrogen to donate a hydrogen bond to either Water-3 or Thr-242 the geometry remains plausible (blue circle).

SUPPORTING REFERENCES

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