RESEARCH ARTICLE

Figures and figure supplements

Single amino acid residue mediates reciprocal specificity in two mosquito odorant receptors

Flavia P Franco *et al*.

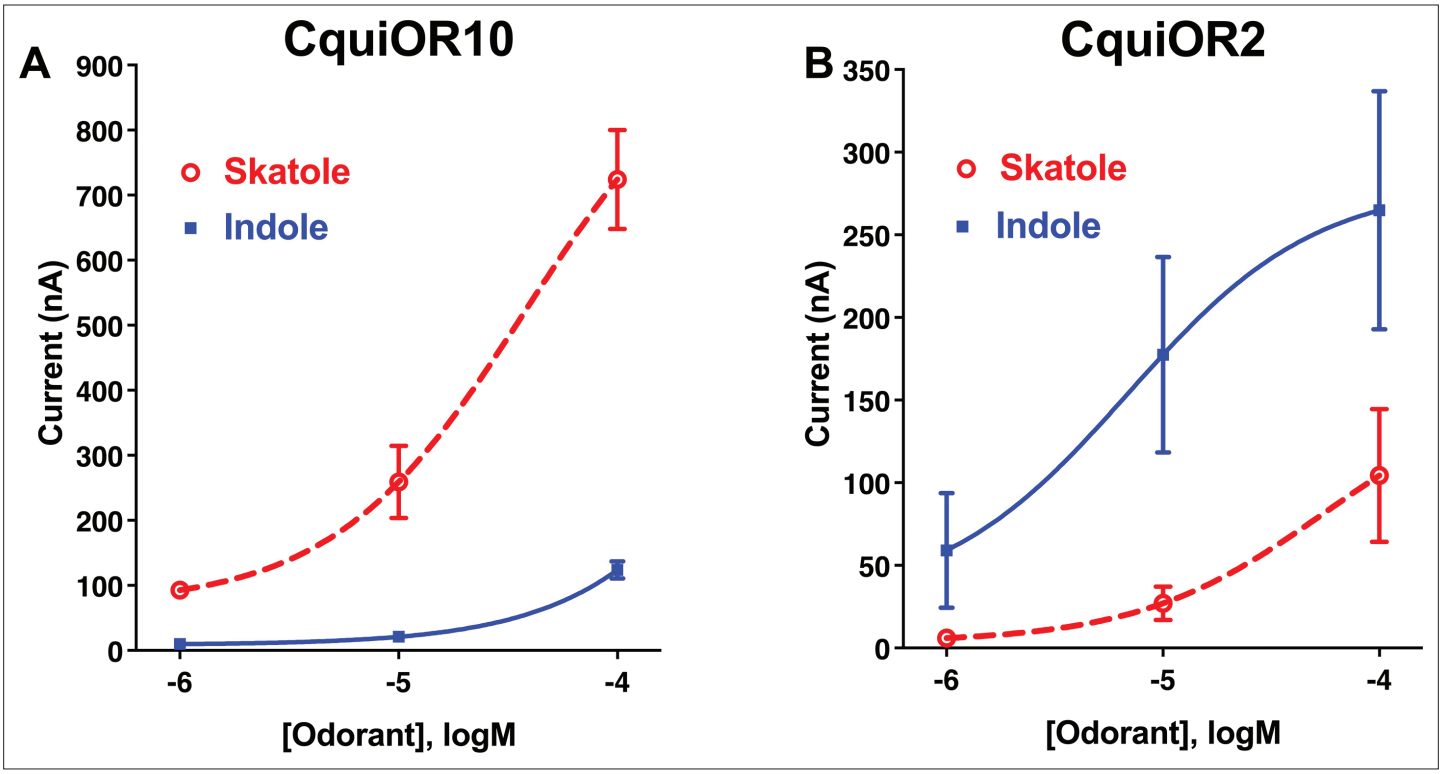

Figure 1. Concentration–response analysis for activation of wildtype odorant receptors (ORs) by skatole and indole. (**A**) CquiOR10 and (**B**) CquiOR2. Lines were obtained with nonlinear fit. Bars represent SEM. n = 4–5.

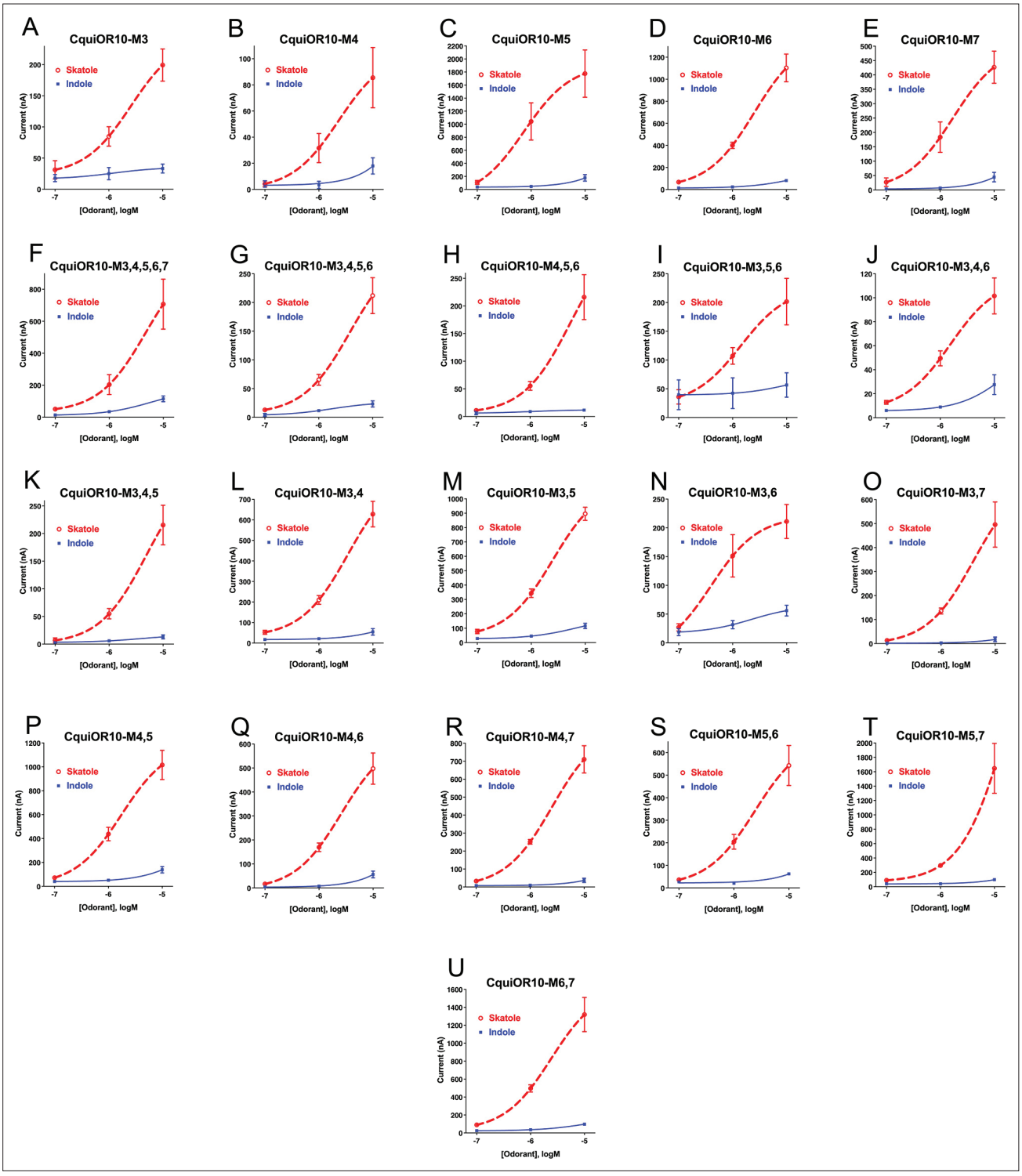

Figure 1—figure supplement 1. Concentration–response analysis for wildtype and chimeric odorant receptors (ORs). (**A**) CquiOR10^{M3}; (**B**) CquiOR10^{M4}; (**C**) CquiOR10^{M5}; (**D**) CquiOR10^{M6}; (**E**) CquiOR10^{M7}; (**F**) CquiOR10^{M3,4,5,6,7}; (**G**) CquiOR10^{M3,4,5,6}; (**H**) CquiOR10^{M4,5,6}; (**I**) CquiOR10^{M3,5,6}; (**J**) CquiOR10^{M3,4,6}; (**K**) CquiOR10^{M3,4,5}; (**L**) CquiOR10^{M3,4}; (**M**) CquiOR10^{M3,5}; (**N**) CquiOR10^{M3,6}; (**O**) CquiOR10^{M3,7}; (**P**) CquiOR10^{M4,5}; (**Q**) CquiOR10^{M4,6}; (**R**) CquiOR10^{M4,7}; (**S**) CquiOR10^{M5,6}; (**T**) CquiOR10^{M5,7}; and (**U**) CquiOR10^{M6,7}. Lines were obtained with nonlinear fit. Barsrepresent SEM. $n = 3$.

A

```
CqOR10    MTAAPILDCPIISVNVRVWHFWSFVLKHDAMRY-ISIIPVGVMNVFMFADLYR---AWGN  56
CqOR2     M---QIEDCPIIGVNVRVWLFWAYLRERKWLSYLLGCIPVTVLNVFQFMNLFHIILSGGT  57
          *    * *****.****** **::: ::. : * :. *** *:*** * :*::   : *.

CqOR10    IDEVIINAYFAMIFFNAVLRTIFILCNRQDYEDFLQRIAEVYSEIAMIDDHVVQKLVRKF  116
CqOR2     MNKIIIDGYFTVLYFNLVLRTTFLLTNRSKFEQFFEGIAAEYAKLEKRND--IRLLLEQL  115
          ::::**:.**:::::** **** *:* **..:*:*:: **  *:::   :*  :: *:.::
CqOR10    TKRARLLSKANLVLGAVISTCYVVYPLFTGTRSLPYGMFIPGVNNFKTPLYQVFFIGQAV  176
CqOR2     TRRARILSKSNLWLGAFISACFVTYPLFSPDSGLPYGVYVPGVNMQSSPTYEIVFFLQVY  175
          *:***:***:** ***.**:*:*.****:   .****:::****  .:* *::.*: *.
CqOR10    LTFPGCCMYIPFTSFFATTTLFGLVQIQTLQRQLRTFKDEVVKENRALVESKLEKCIEDH  236
CqOR2     LTFPACCMYIPFTSFYCTCTLFGLVRIAALKESLERLHQFSS--EPKTLLAKVKECLQYH  233
          ****.**********:.* ******:* :*:..*. :::     :   : :*:::*:: *
CqOR10    KRIIRYVSDVNSLVTYICLIEFMSFGLMLCALLFLLNIIENPAQIIIVVAYIFMIISQIF  296
CqOR2     KKIIKYIRDLNELVTFIFLLELLSFGMMLCALLFLLSTSNQLAQMVMIGSYIFMILSQMY  293
          *:**:*: *:*.***:* *:*::***:*********.  :: **:::: :*****:**::
CqOR10    TFYWHANELREESMGIAEAA-YDAPWVELDDSMKKKLLLIIARAQQPLEITVGNVYAMTL  355
CqOR2     ALYWHSNDVLEQSLKIADSLYYNGNWLKFSTPVKKLMILMIARAQRPLVIKVGNVYPMTL  353
          ::***:*:: *:*: **::  *:. *:::.  :** ::*:*****:** *.***** ***
CqOR10    EMFQSLLNASYSYFTLLRRVYN    377
CqOR2     EMFQKLINASYSYFTLLRRVYN    375
          ****.*:***************
```

B

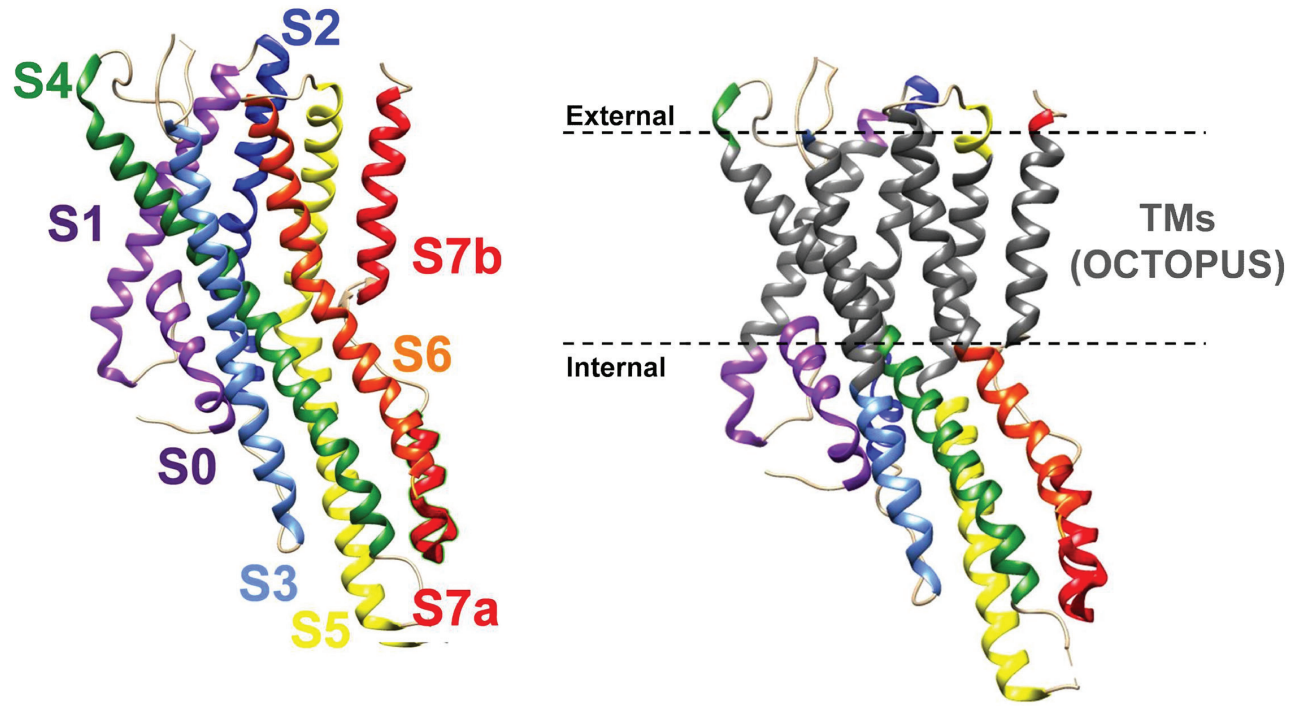

Figure 2. Alignment of the amino acid sequences of CquiOR10 and CquiOR2 highlighting the predicted transmembrane (TM) domains and a comparison of predicted and experimentally determined TM domains of the odorant receptor coreceptor, AbakOrco. (**A**) CqOR10 and CqOR2 are abbreviations for CquiOR10 and CquiOR2, respectively. The TM domains, predicted by OCTOPUS, are displayed in red and blue for CquiOR10 and CquiOR2, respectively. The sequences of the N-terminus and the intracellular loops are displayed in black, and the C-terminus and extracellular loops

Figure 2 continued on next page

Figure 2 continued

in green. (**B**) Left: the cryo-EM structure of AbakOrco (PDB, 6C70) displayed in rainbow color using UCSF Chimera (***Pettersen et al., 2004***). Right: the predicted TM domains (right) are displayed in gray. The dashed lines represent the membrane boundaries.

Figure 3. Concentration–response curves obtained with chimeric odorant receptors (ORs) stimulated with skatole and indole. (**A**) CquiOR10^{M2,7}; (**B**) CquiOR10^{M2,5,6,7}; (**C**) CquiOR10^{M2,5,6,7}_Outer; (**D**) CquiOR10^{M2,5,6,7}_Mid;Inner; (**E**) CquiOR10^{M2,5,6,7}_Inner; (**F**) CquiOR10^{M2,5,6,7}T78I; (**G**) CquiOR10^{M2,5,6,7}L73A; (**H**) CquiOR10^{M7}A73L; (**I**) CquiOR10A73L; (**J**) CquiOR2L74A. Lines were obtained with nonlinear fit. Bars represent SEM. The number of replicates (n) were 7, 4, 5, 5, 4, 3, 9, 7, 6, and 5, respectively.

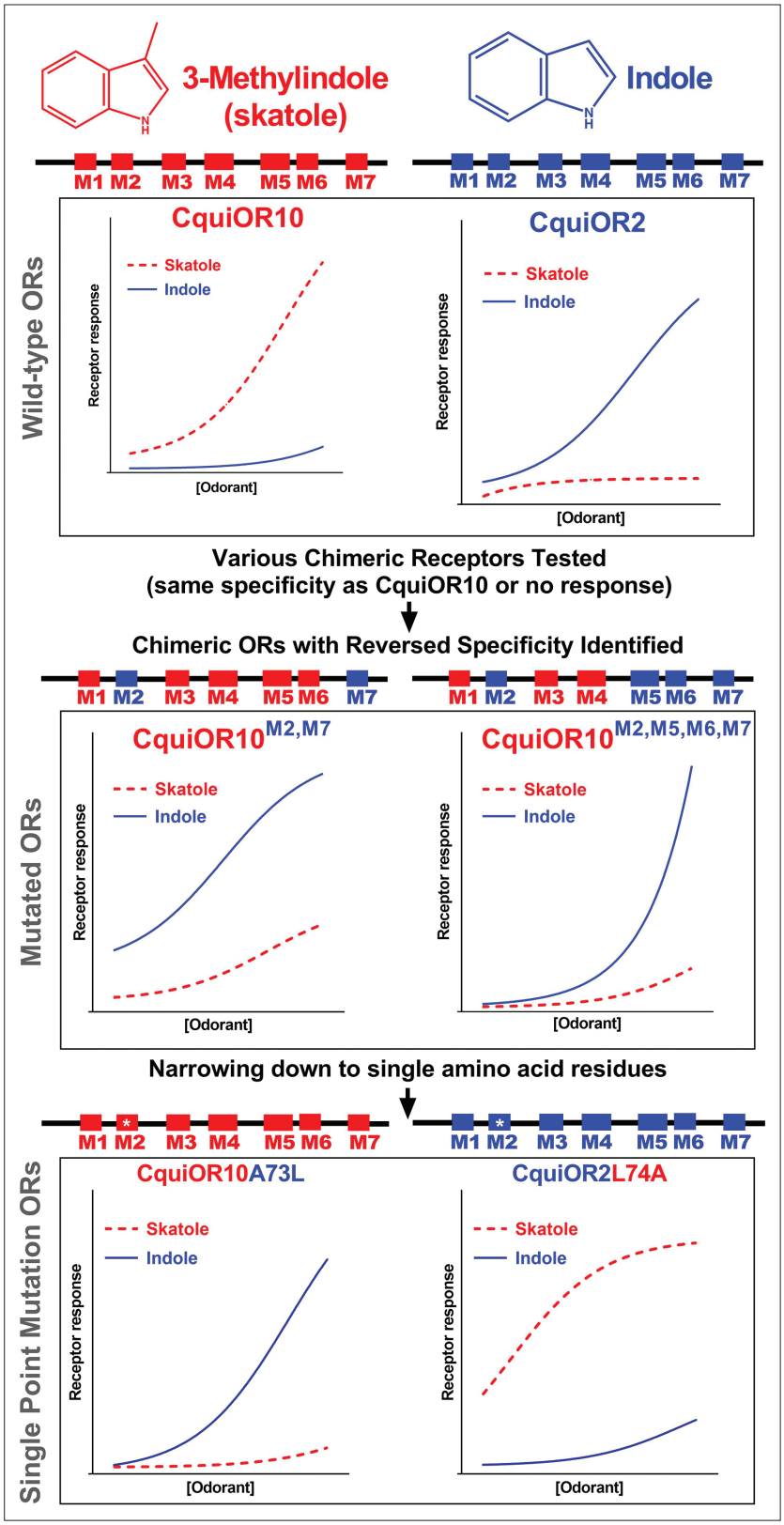

Figure 3—figure supplement 1. Schematic view of the workflow.

	57 ↓ 73 ↓ 116 ↓	Selective to
CquiOR10	IDEVIINAYFAMIFFNAVLRTIFILCNRQDYEDFLQRIAEVYSEIAMIDDHVVQKLVRKF	Skatole
CquiOR10M^{2,5,6,7}	IDKIIIDGYFTVLYFNLVLRTTFILCNRQDYEDFLQRIAEVYSEIAMIDDHVVQKLVRKF	Indole
	Outer Mid Inner	
CquiOR10M^{2,5,6,7}_Outer	IDEVIINAYFTVLYFNLVLRTTFILCNRQDYEDFLQRIAEVYSEIAMIDDHVVQKLVRKF	Indole
CquiOR10M^{2,5,6,7}_Mid+Inner	IDKIIIDGYFAMIFFNAVLRTIFILCNRQDYEDFLQRIAEVYSEIAMIDDHVVQKLVRKF	Skatole
CquiOR10M^{2,5,6,7}_Inner	IDKIIIDGYFTVLYFNAVLRTIFILCNRQDYEDFLQRIAEVYSEIAMIDDHVVQKLVRKF	Skatole
CquiOR10M^{2,5,6,7}_T78I	IDKIIIDGYFTVLYFNLVLRTIFILCNRQDYEDFLQRIAEVYSEIAMIDDHVVQKLVRKF	Indole
CquiOR10M^{2,5,6,7}_L73A	IDKIIIDGYFTVLYFNAVLRTTFILCNRQDYEDFLQRIAEVYSEIAMIDDHVVQKLVRKF	Skatole

Figure 4. Partial sequences of CquiOR10 and chimeric odorant receptors (ORs) highlighting transmembrane domain-2 (TM2). The two last residues of the extracellular loop-1 (Ile-57 and Asp-58) appear in the N-terminus. The TM2 was divided into the arbitrary segments outer, middle (mid), and inner to identify specificity determinants.

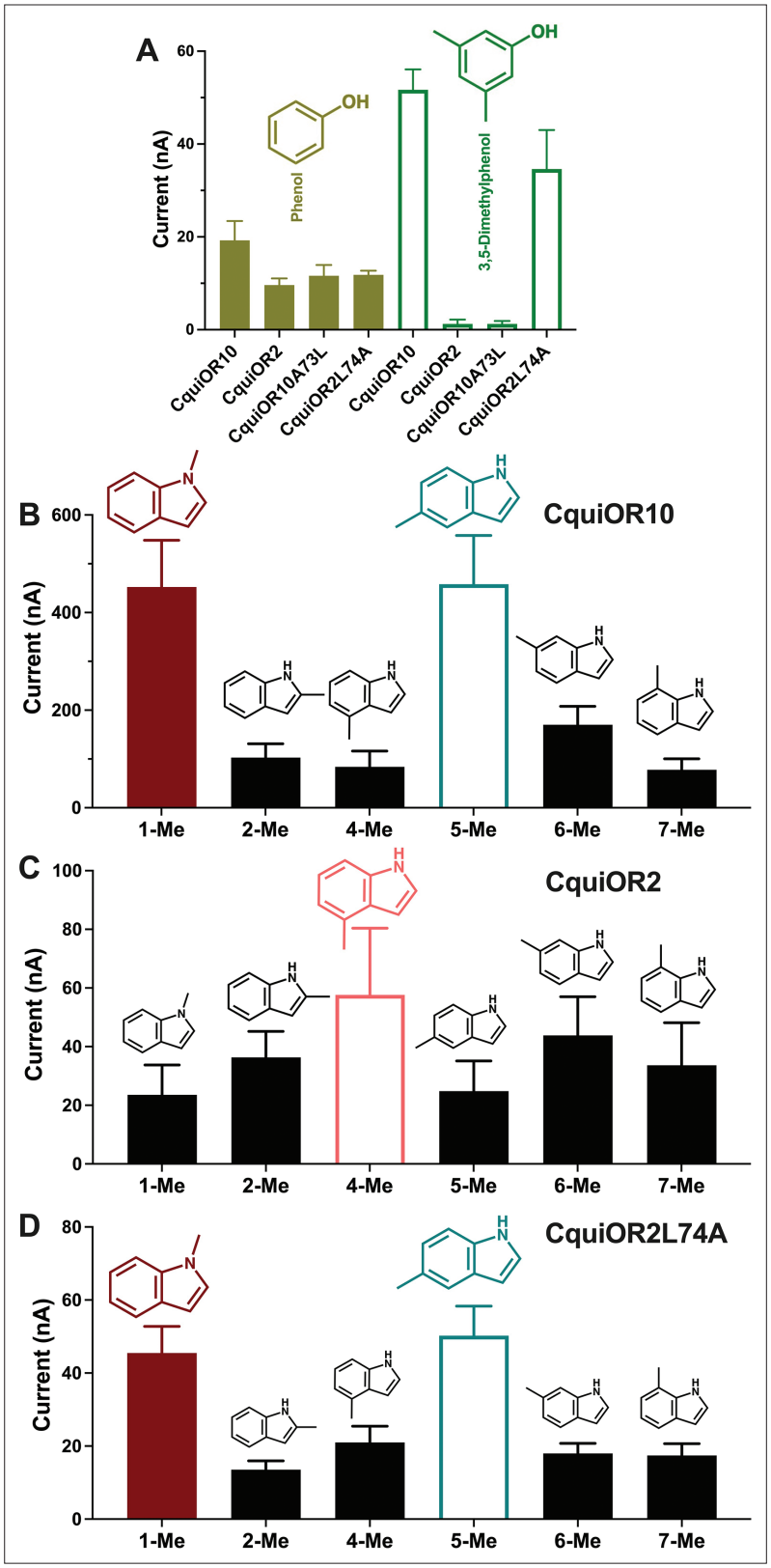

Figure 5. Quantification of wildtype and chimeric receptors to phenol and 2,3-dimethylphenol, and methylindoles. (**A**) Each receptor was co-expressed with CquiOrco in *Xenopus* oocytes and stimulated with the phenolic compounds at 1 mM. n = 3–5. (**B**) CquiOR10/CquiOrco-, (**C**) CquiOR2/CquiOrco-, and (**D**)-CquiOR2L74A-expressing oocytes were stimulated with 100 μM of the specified methylindoles. n = 9–11. Bars represent SEM.

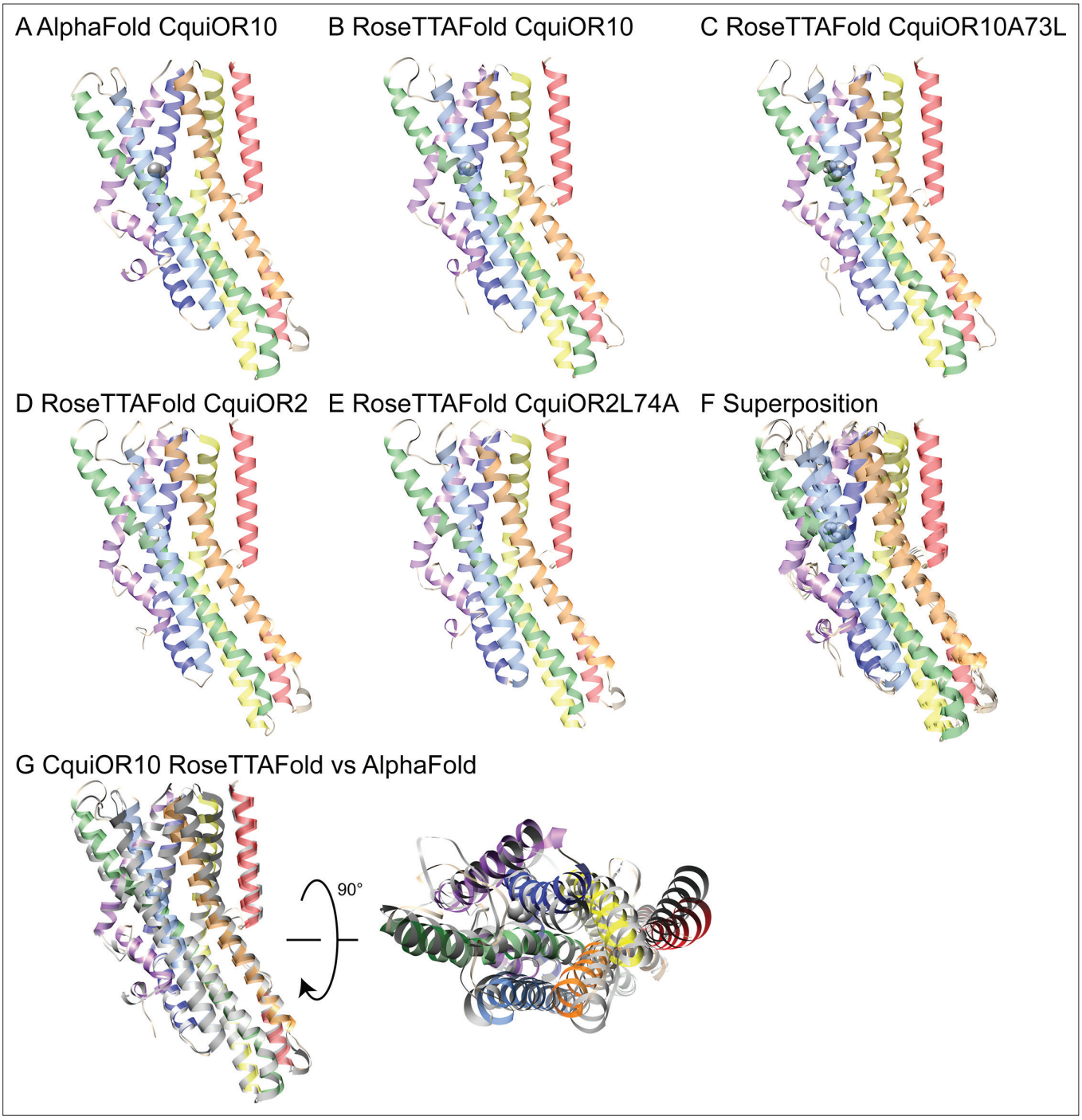

Figure 6. AlphaFold and RoseTTAFold models. Structural models of CquiOR10 (**A, B**), CquiOR10A73L (**C**), CquiOR2 (**D**), and CquiOR2L74A (**E**) with AlphaFold (**A**) and RoseTTAFold (**B–E**) structure prediction methods. Superposition of all RoseTTAFold models (**F**) resulted in transmembrane helix root mean square deviation (RMSD) of 0.8 Å when aligned with RoseTTAFold CquiOR10. (**G**) The transmembrane helix RMSD of CquiOR10 RoseTTAFold (rainbow) vs. AlphaFold (gray) was 1.7 Å. Loops were not included in RMSD calculation due to inherent flexibility during structure prediction.

```
CquiOR10          1 --------------------------------------------------      0

MhraOR5           1 GPGRAKIDVDSVDHTDDYIHLRKWIKRIGIILRISGHWPFRLPHEKRNQH     50

CquiOR10          1 --------------------------------------------------      0

MhraOR5          51 KSKFRQVYSCLVITLGFITCSCYCIGLCLSESIAQALNNITVTSYFLQSC    100

CquiOR10          1 -----------------------------------MTAAPILDCPIISVN     15
                                                      .....:..|..::.:
MhraOR5         101 VCYVSFIINSRKLETLFNYLFENEVVGCPRGYKMSSIKTTLFRCKFVAFS    150

CquiOR10         16 VRVWHFWSFVLKHDAMRYISIIPVGVMNVFMFAD---------LYRAWGN     56
                    :.:..|:.:::       .:::|:.|:.|...|.         ...||..
MhraOR5         151 LGILSFFGWLM-------WTLLPLAVLVVDSGATGGGNQTSLRFVEAWYP    193

CquiOR10         57 ID-------EVI-INAYFAMIFFNAV---------LRTIFIL----CNRQ     85
                    .|       ||| |....||||....         :..|||:    |...
MhraOR5         194 FDTTTSPMNEVIAIYEAVAMIFLITAPMSSDIMFCVLMIFIVEHLKCLGM    243

CquiOR10         86 DYEDFLQRIAEVYSEIAMIDDHVVQKLVRKFTKRARLLSKANLVLGAVIS    135
                    ..|..|:.|:....:....||.|....|::           .:|:|.
MhraOR5         244 AIECTLKGISTNQHQNIGFDDSVSDVNVQR-----------RIVIGK---    279

CquiOR10        136 TCYVVYPLFTGTRSLPYGMFIPGVNNFKTPLYQVFFIGQAVLTFPGCCMY    185
                                                ::|:..:               :
MhraOR5         280 ---------------------------ESPIQSI---------------H    287

CquiOR10        186 IPFTSFFATTTLFGLVQIQTLQRQLRTFKDEVVKENR-----ALVES---    227
                    :|                |:...||   ..|.|.:|.|      .::.|
MhraOR5         288 VP---------------IKECSRQ---SSDAVFREKRHGTHHQIIRSHNY    319

CquiOR10        228 ----KLEKCIEDHKRIIRYVSDVNSLVTYICLIEFMSFGLMLCALLFLLN    273
                        .|...::.|.:|.|.:..|.|:.:.....|.:..|.:|||.:.|.
MhraOR5         320 TDATSLCNIVDSHVKIYRTMEIVQSVYSSYFATLFFTSCLAVCALAYFLA    369

CquiOR10        274 IIENP-AQIIIVVAYIFMIISQIFTFYWHANELREESMGIAEAAYDAPWV    322
                    ..... .::..:|.|:..|..:||.....|.|:.|:.:.:..|.|.:..|
MhraOR5         370 ATSTSFTRVPGMVLYLMYIFLRIFLLCLLATEVAEQGLNLCHAGYSSKLV    419

CquiOR10        323 ELDDSMKKKLLLIIARAQQPLEITVGNVYAMTLEMFQSLLNASYSYFTLL    372
                    ...|.::..:..|..|||.||.||....:.:.|....|:.....:||.:|
MhraOR5         420 LASDHVRSTIQAIATRAQIPLSITGARFFTVNLSFLASMAGVMLTYFIVL    469

CquiOR10        373 RRVYN-----    377
                    .:| |
MhraOR5         470 LQV-NAKPKP    478
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Figure 6—figure supplement 1. Pairwise alignment of CquiOR10 and MhraOR5.

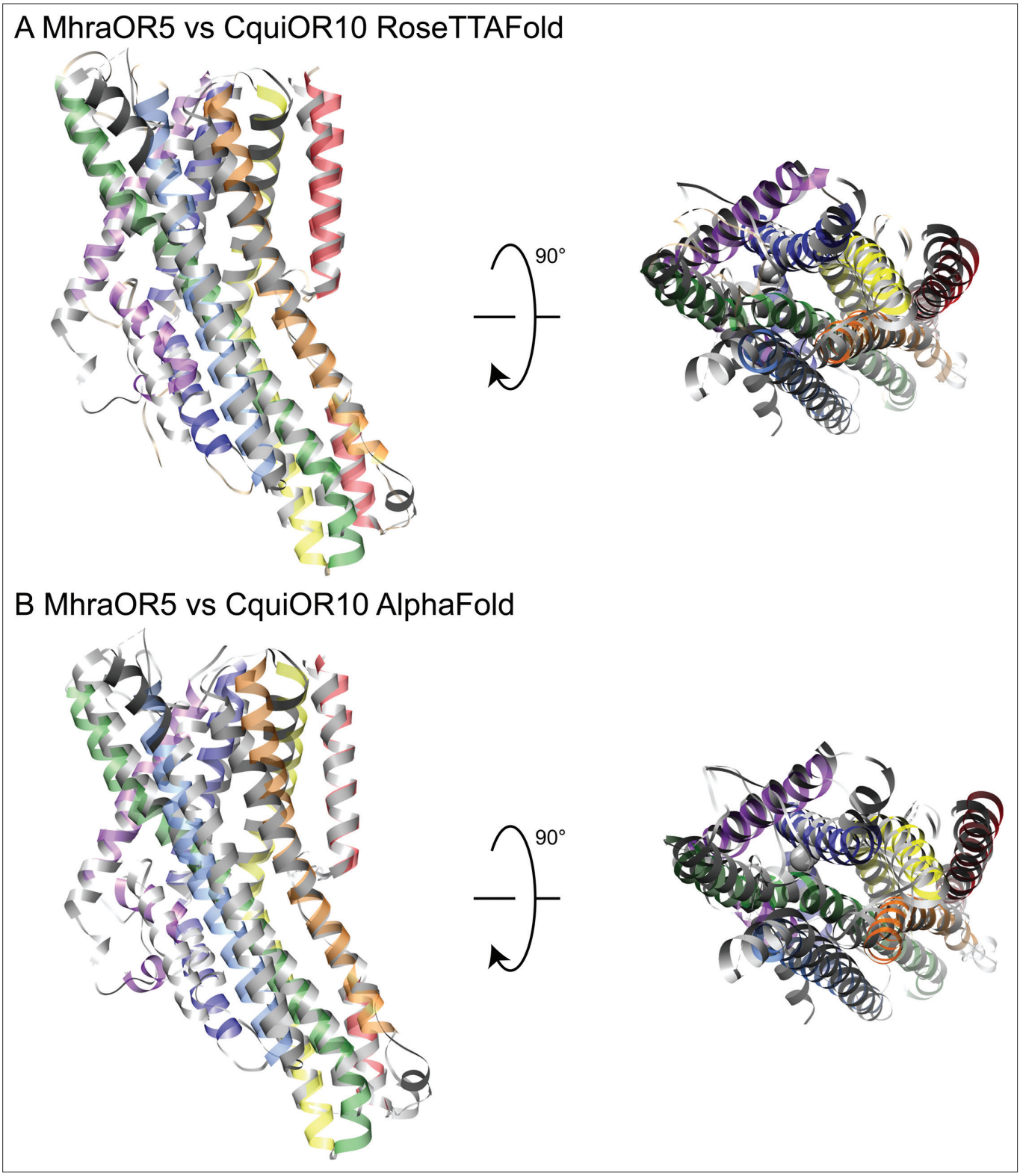

Figure 6—figure supplement 2. Overlay of MhraOR5 structure and CquiOR10 models. (**A**) RoseTTAFold and (**B**) AlphaFold models for CquiOR10.

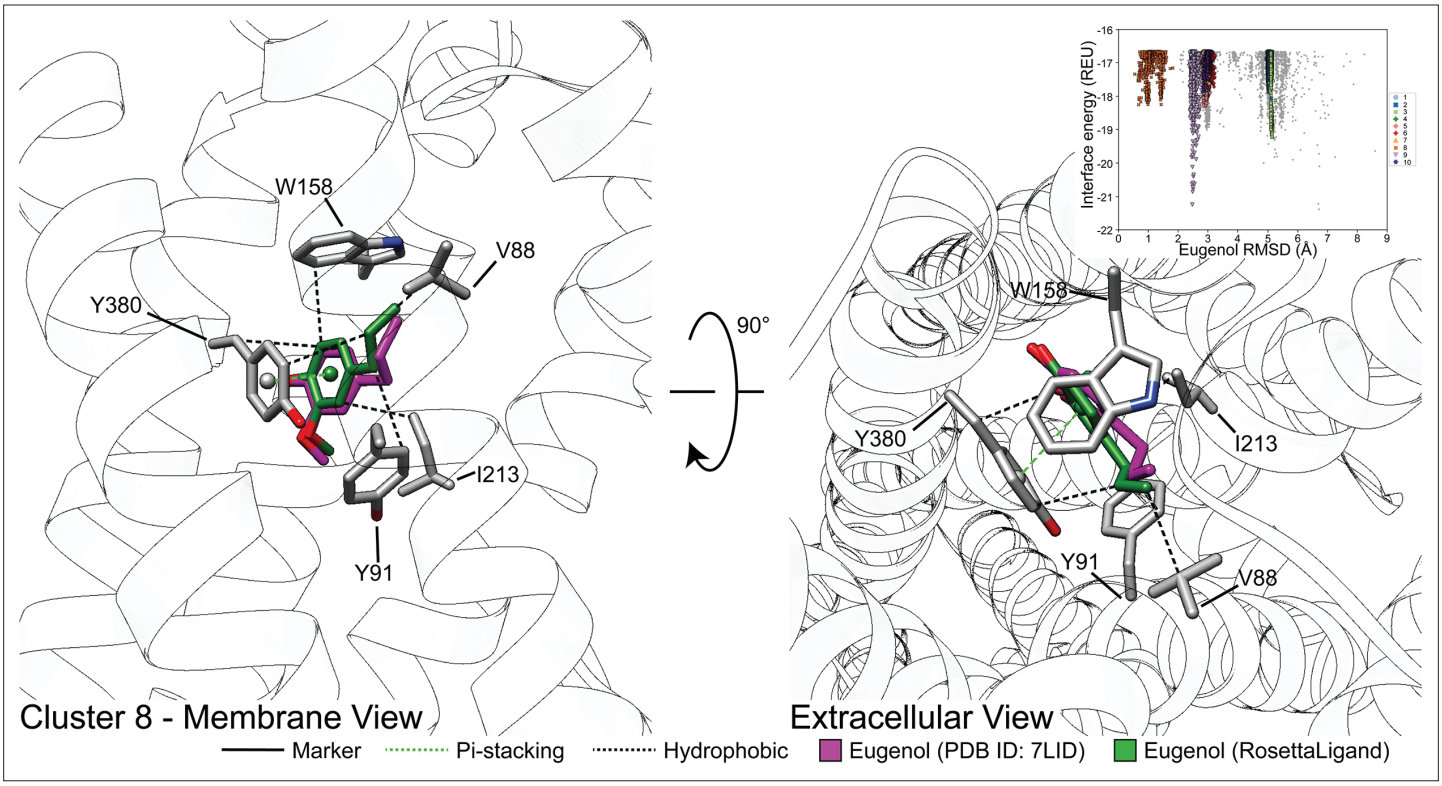

Figure 6—figure supplement 3. Representative model of docked eugenol with MhraOR5.

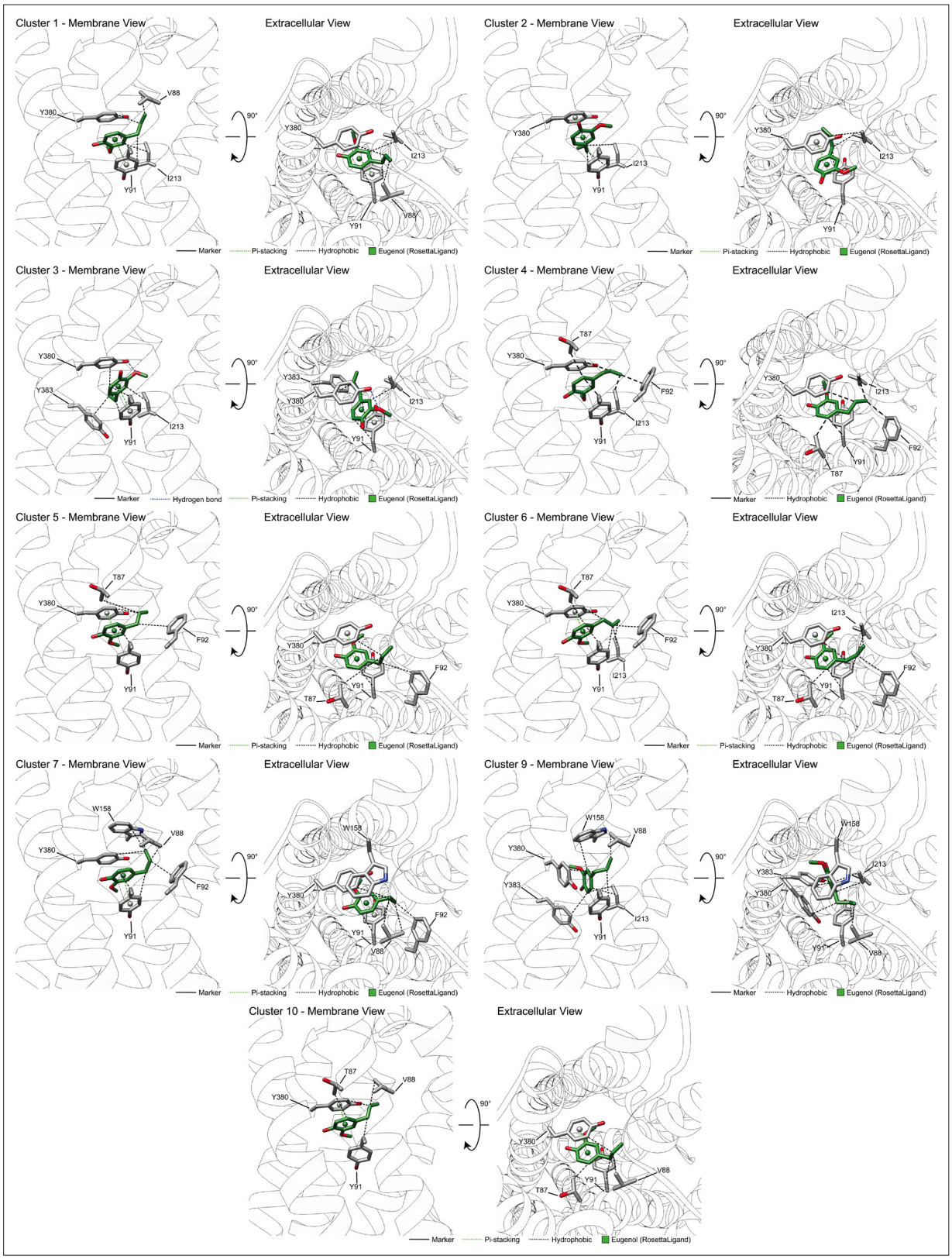

Figure 6—figure supplement 4. Additional clusters of eugenol docked to MhraOR5.

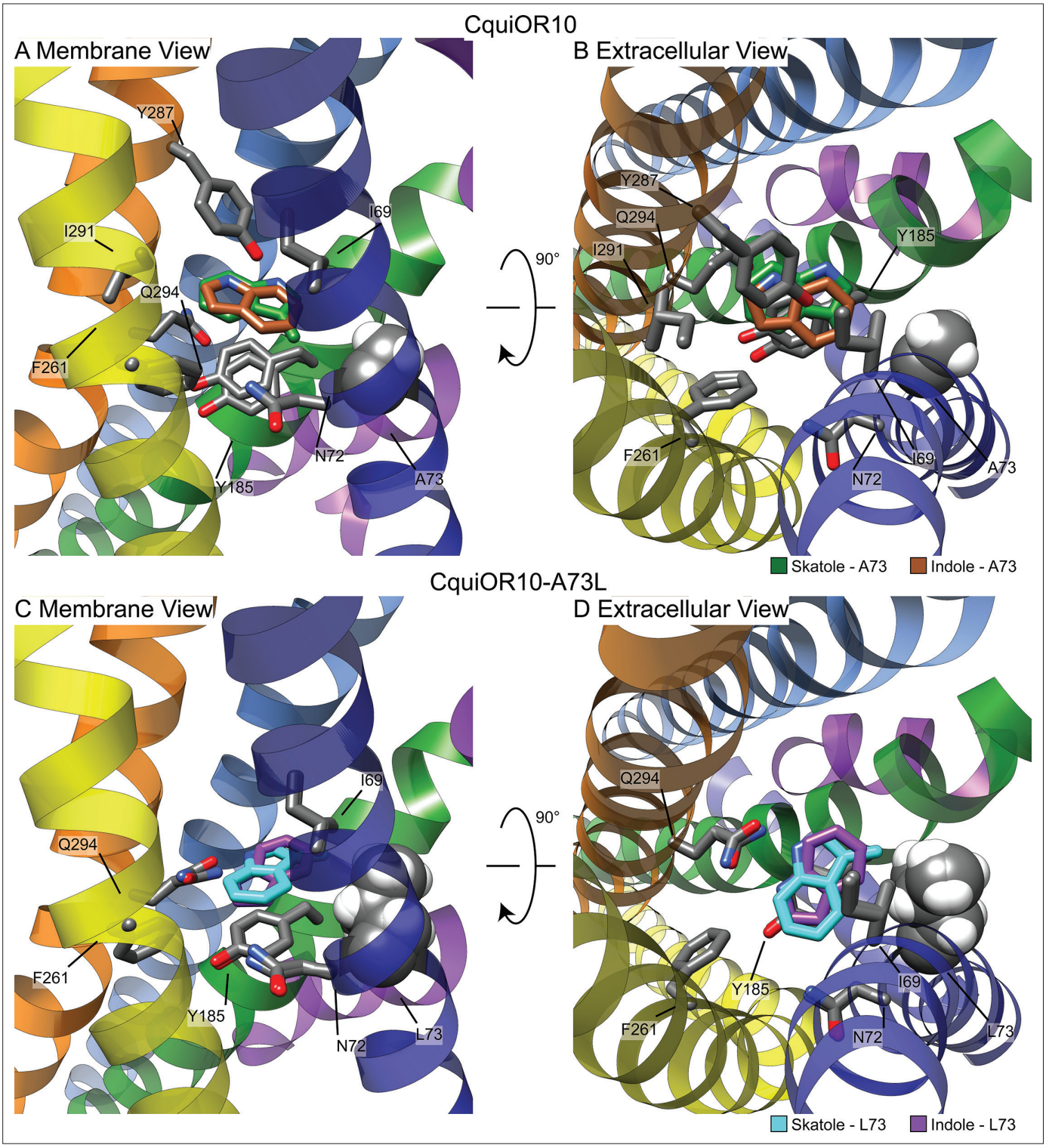

Figure 7. Representative models of docked skatole and indole in complex with CquiOR10 and CquiOR10A73L using RosettaLigand. Each model shown is the lowest interface-energy model from the 10 largest clusters of each docking study. CquiOR10 – skatole (forest green), CquiOR10 – indole (brown), CquiOR10A73L – skatole (light blue), and CquiOR10A73L – indole (purple). Atoms that are not indole/skatole carbon atoms are color-coded by atom type: carbon (gray), nitrogen (dark blue), and oxygen (red). Ala-73 and Leu-73 indicated with space-filling representation. (**A, B**) and (**C, D**) Mebrane and extracellular views for CquiOR10 and CquiOR10A73L, respectively.

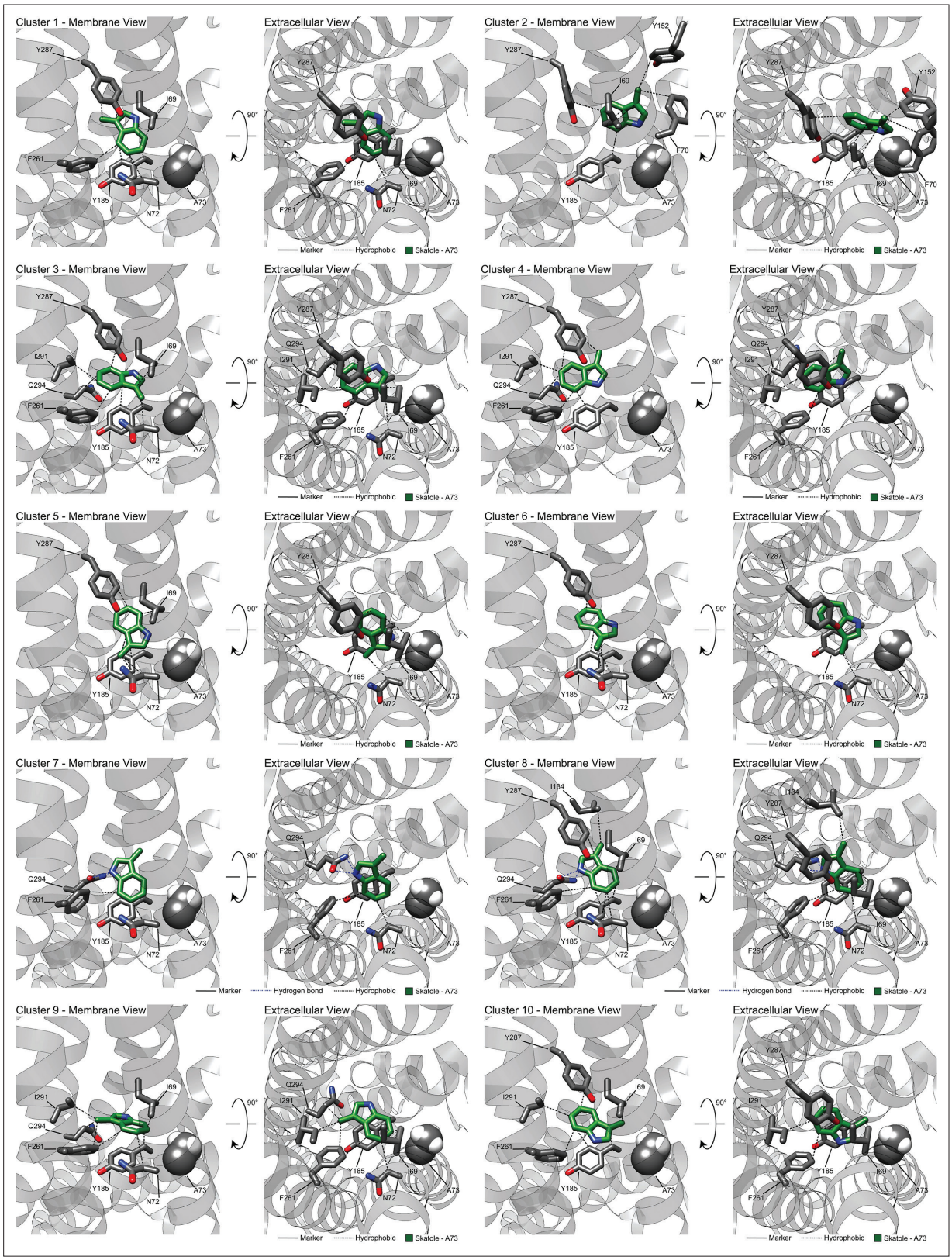

Figure 7—figure supplement 1. Clusters from RosettaLigand docking of skatole or indole to CquiOR10 or CquiOR10A73L.

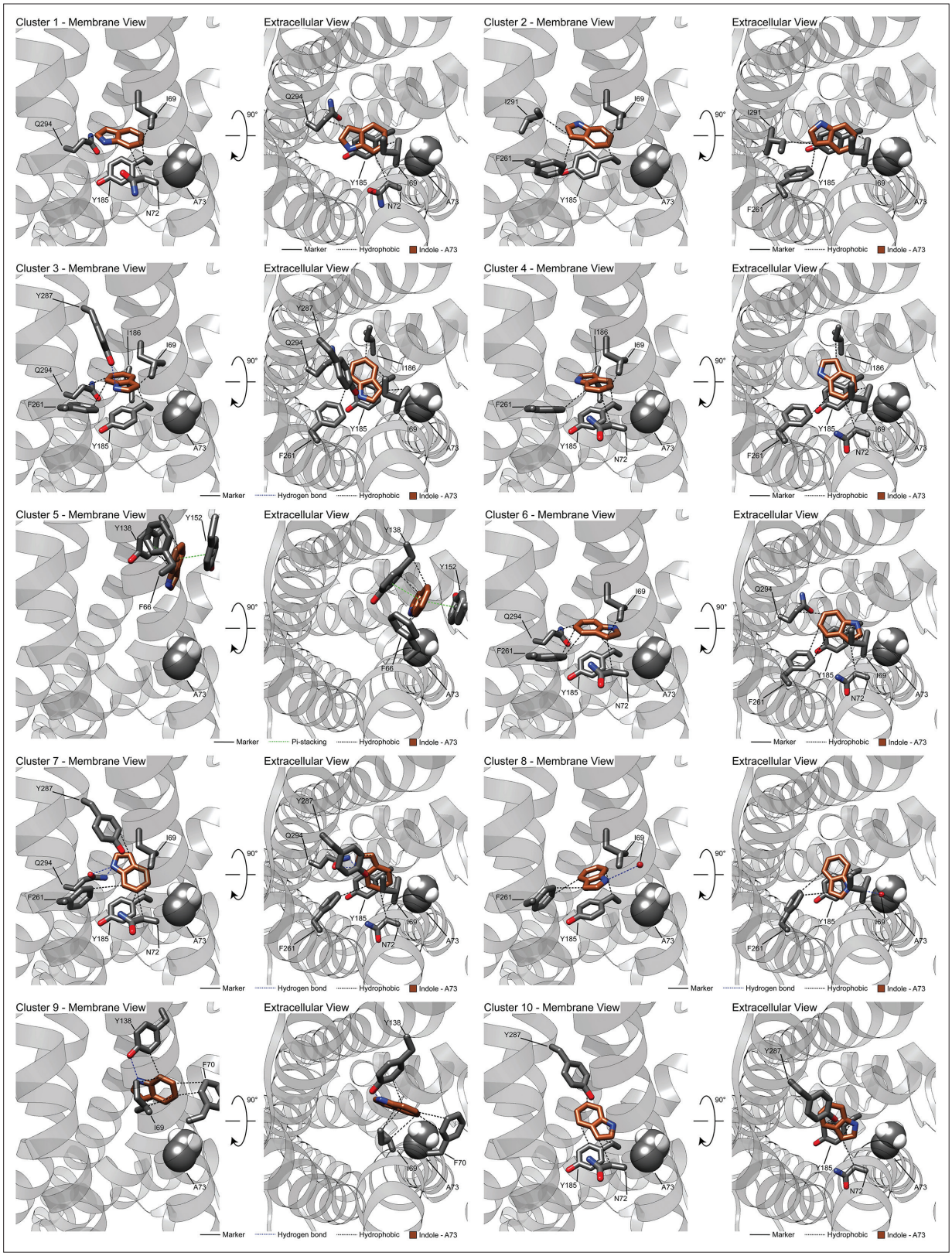

Figure 7—figure supplement 2. Zoom-out view of CquiOR10 or CquiOR10A73L complexed to skatole or indole.

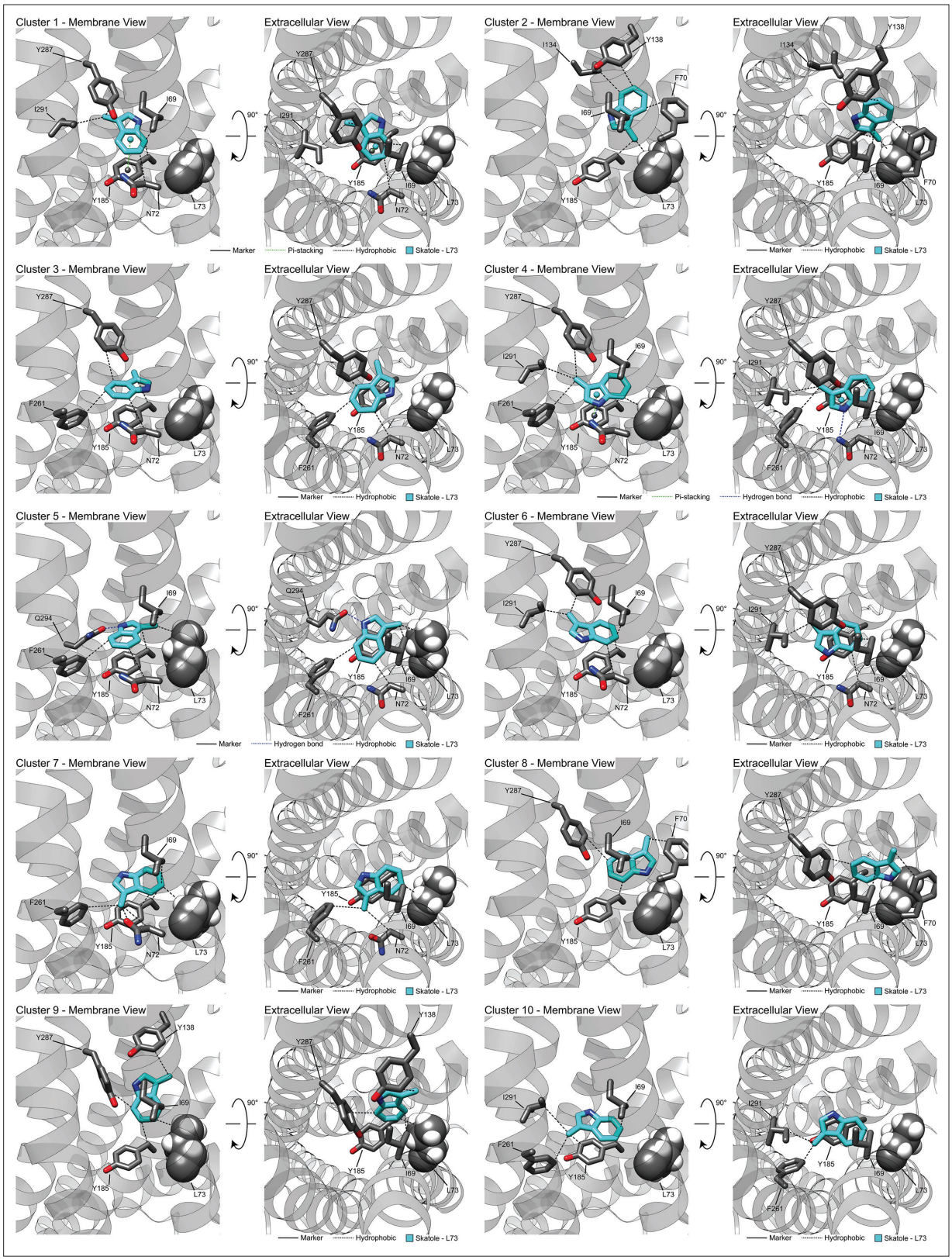

Figure 7—figure supplement 3. Representative models of docked skatole or indole to CquiOR10 or CquiOR10A73L with PLIC analysis.

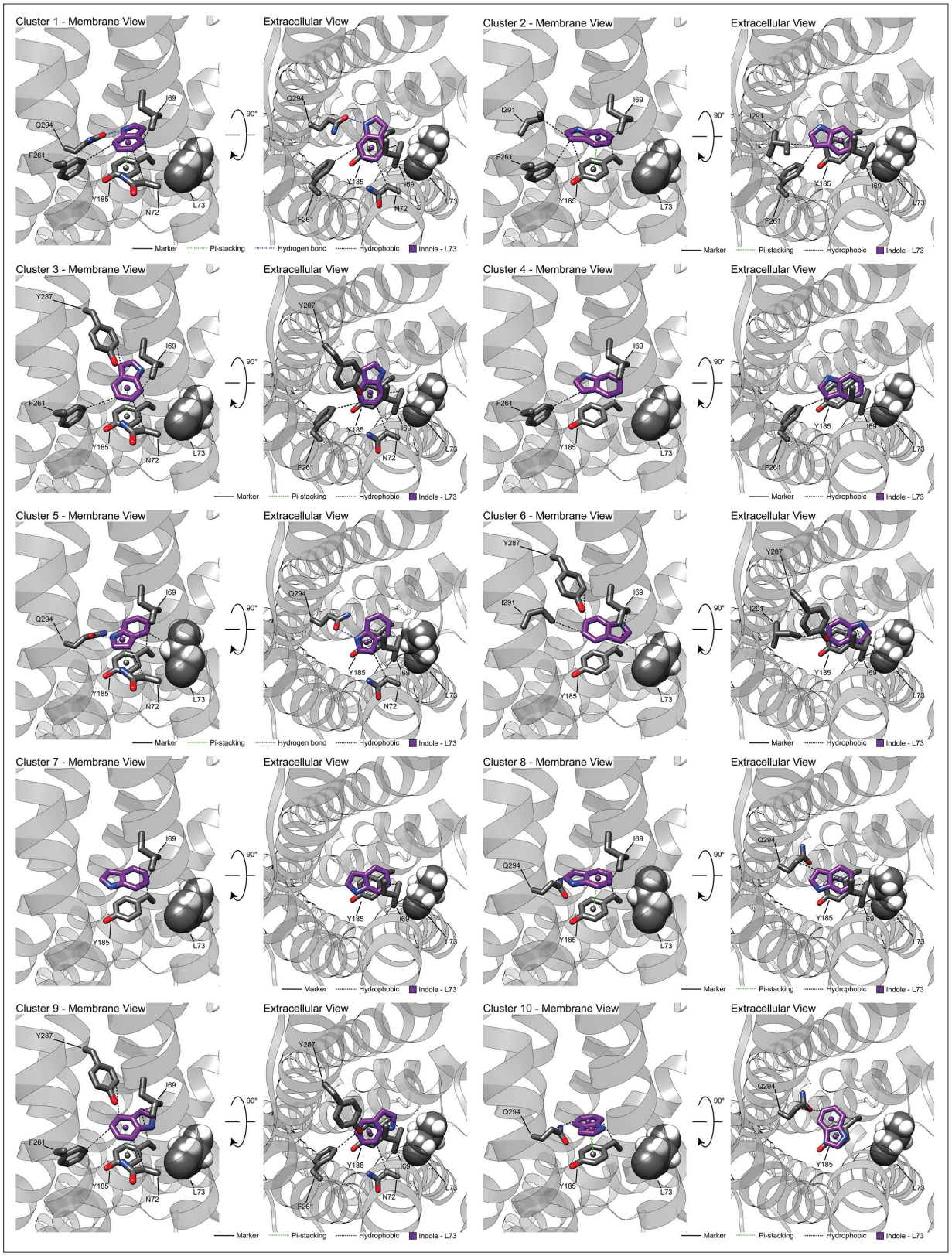

Figure 7—figure supplement 4. Superimposition of all OR-skatole and OR-indole clusters. OR: odorant receptor.

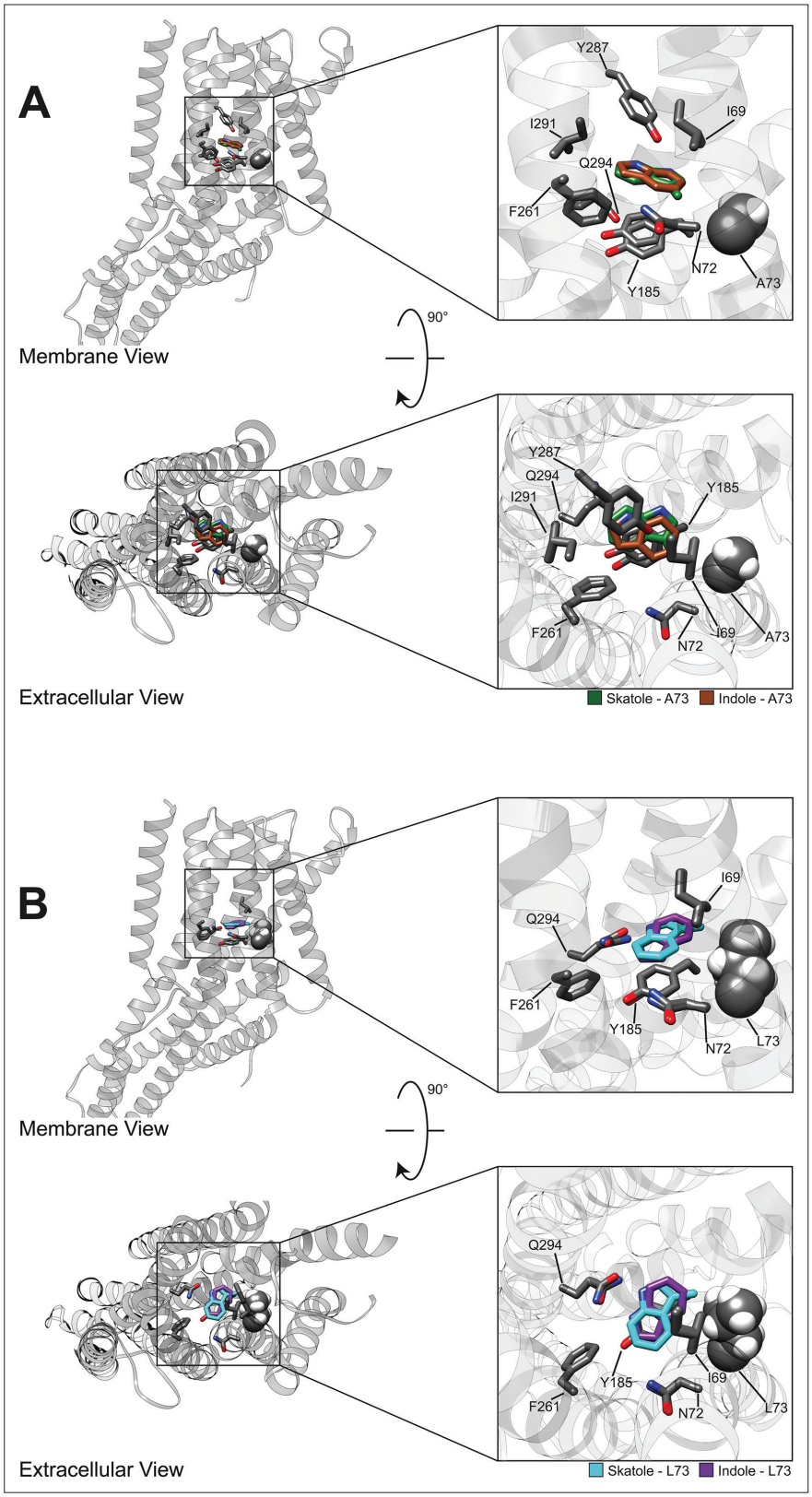

Figure 7—figure supplement 5. Example of sampling from ligand docking. (**A**) CquiOR10 and (**B**) CquiOR10A73L.

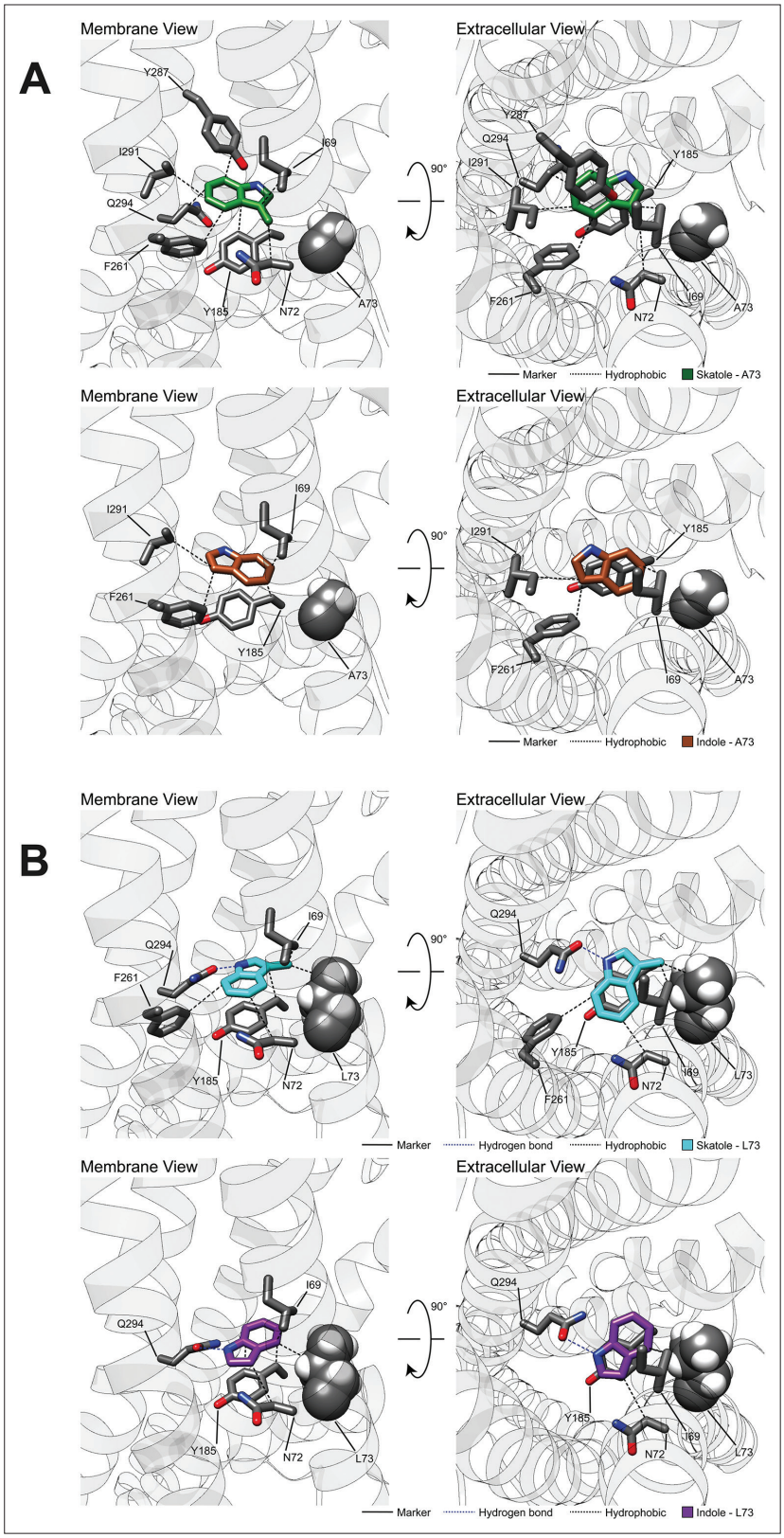

Figure 7—figure supplement 6. Representative RosettaLigand docking of skatole and indole to (**A**) CquiOR10 and (**B**) CquiOR10A73L with PLIP analysis. The representative is the lowest interface-scoring energy model from the 10 most frequent clusters of each test case. Hydrogen bond andpi-stacking interactions were filtered by previously reported bond distances (***Bissantz et al., 2010***).

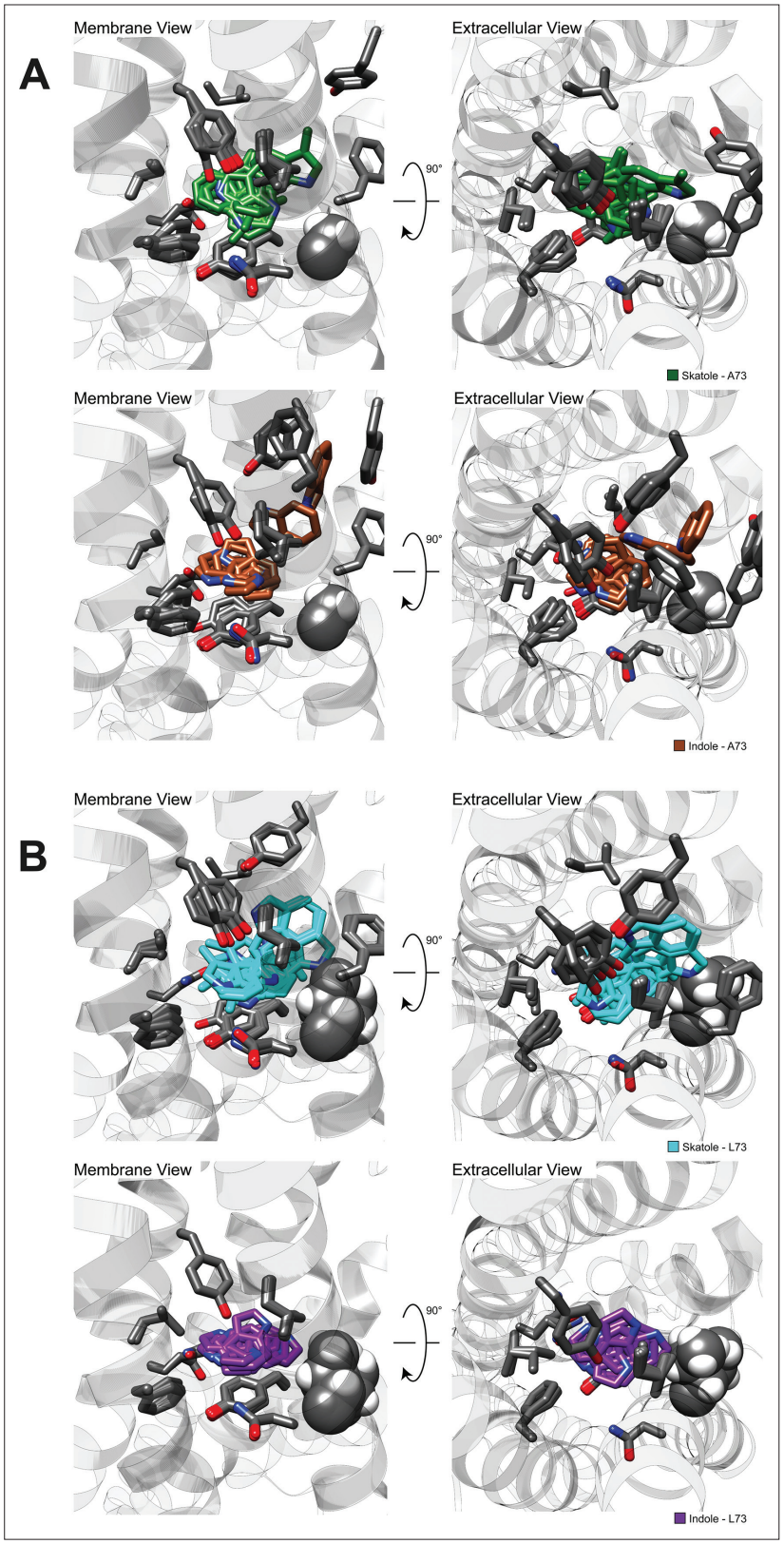

Figure 7—figure supplement 7. Superposition of all clusters of skatole and indole docked to (**A**) CquiOR10 and (**B**) CquiOR10-A73L.

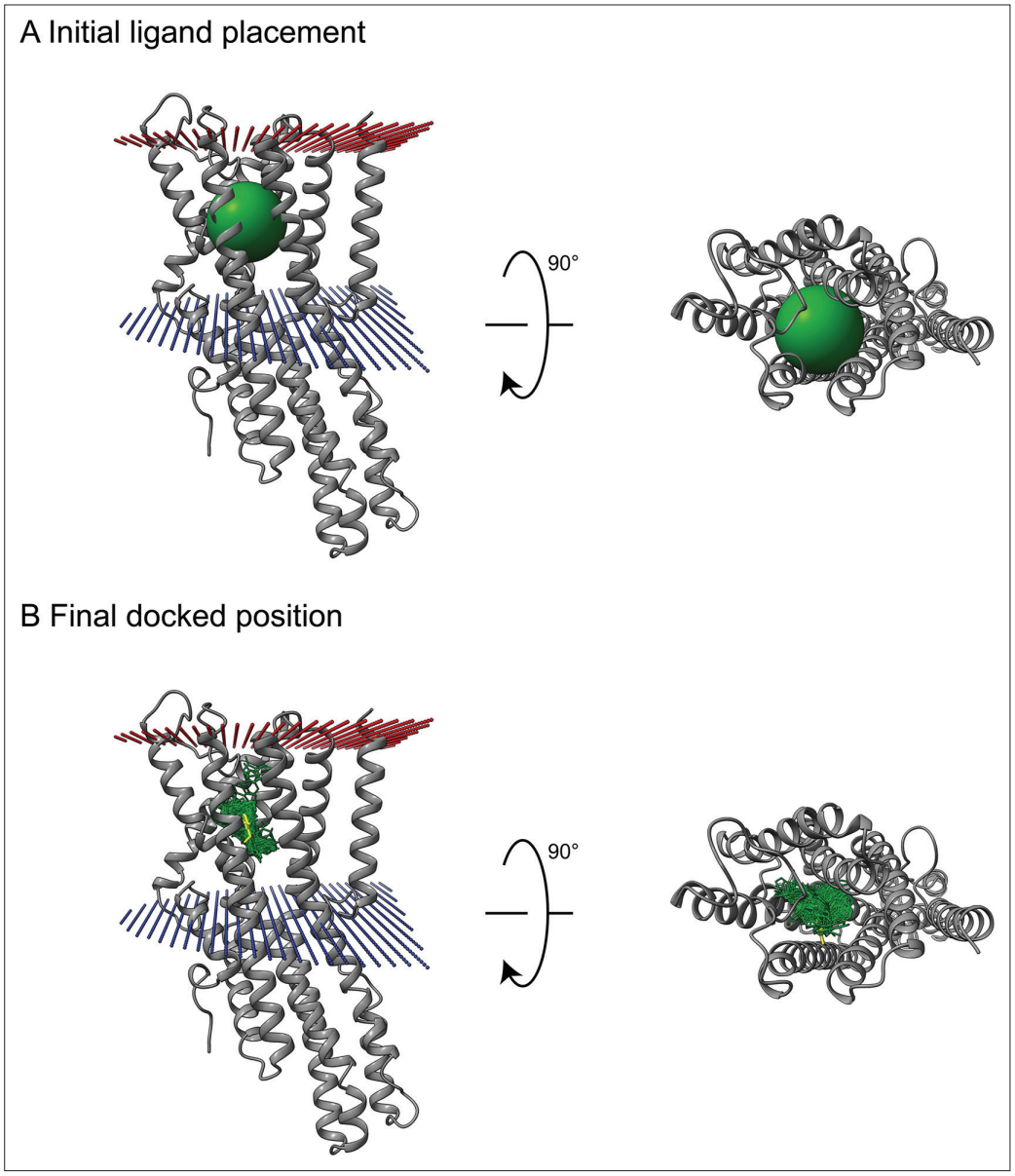

Figure 7—figure supplement 8. Example of sampling from ligand docking. (**A**) Odorants docked to CquiOR (grey) were positioned relative to eugenol from MhraOR5 (PDB 7LID) and then randomlyrotated/translated within 7 Å as a starting position for docking. The green sphere represents the 7 Å sampling boundary. (**B**) An example demonstrating thesampling of skatole (green) docked to CquiOR10 after docking with eugenol (yellow) as a positional reference. Red: extracellular membrane boundary. Blue: cytosplasmic membrane boundary.

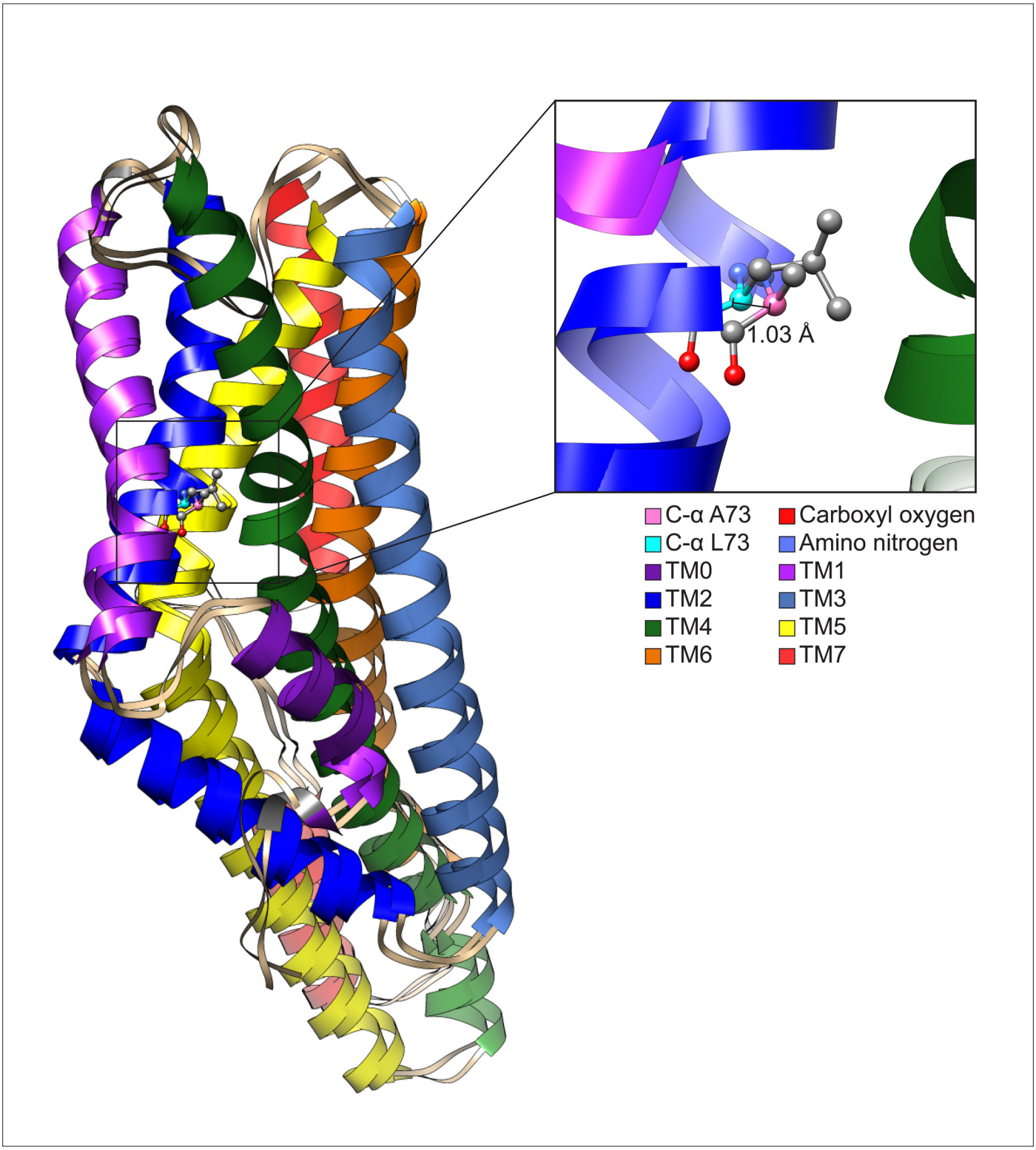

Figure 8. Comparison of CquiOR10 and CquiOR10A73L models. An approximate 1 Å α-carbon outward shift of Leu-73 (forest green) in CquiOR10 model relative to Ala-73 (light blue) in CquiOR10A73L model. Models were superimposed using the TM7b region. Residue 73 amino nitrogen is colored in dark blue, and carboxyl oxygen is colored in red in each model.

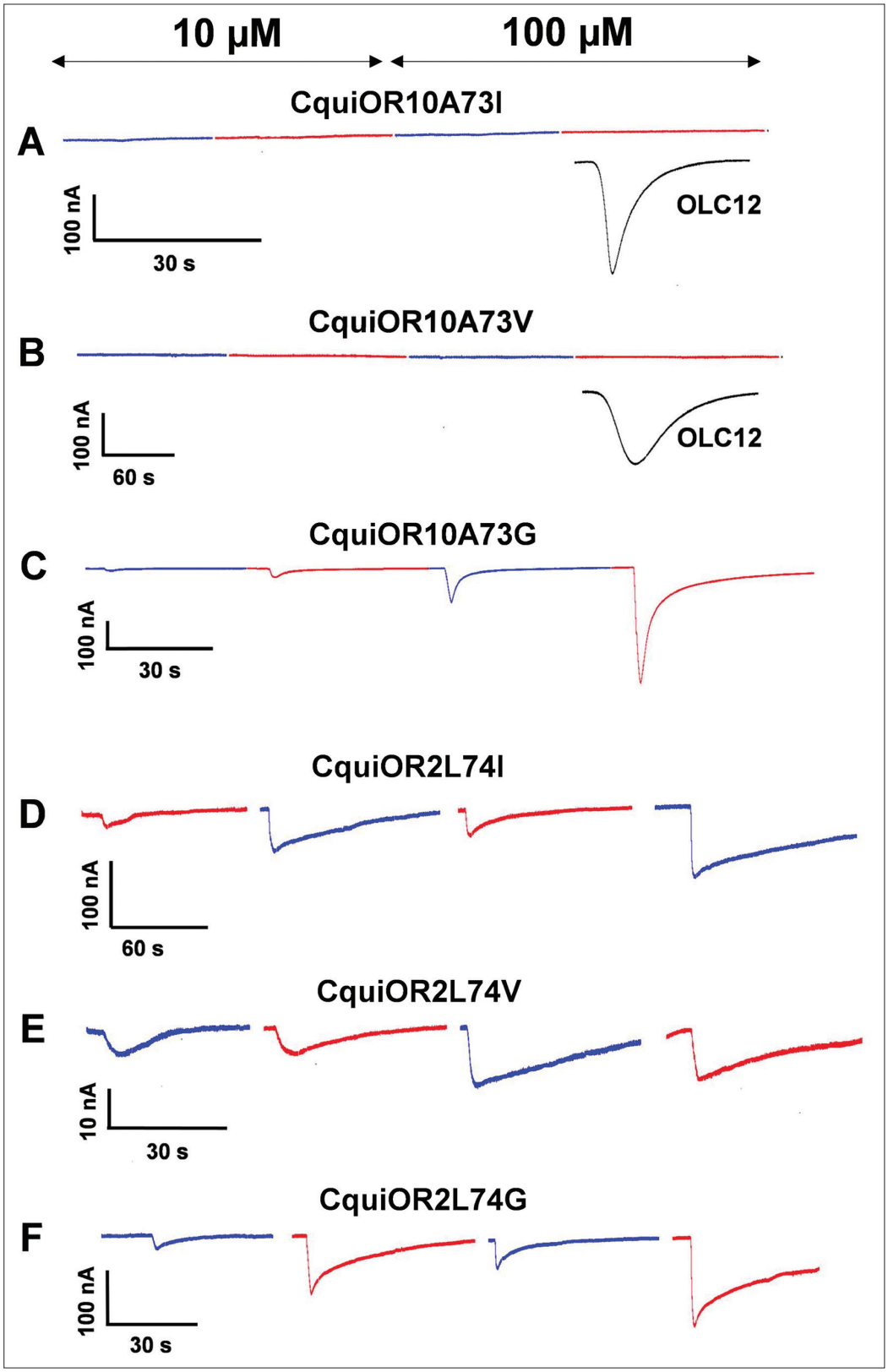

Figure 8—figure supplement 1. Effect of single-point mutations around A73. (**A-C**) CquiOR10 mutants. (**D-F**) CquiOR2 mutants.

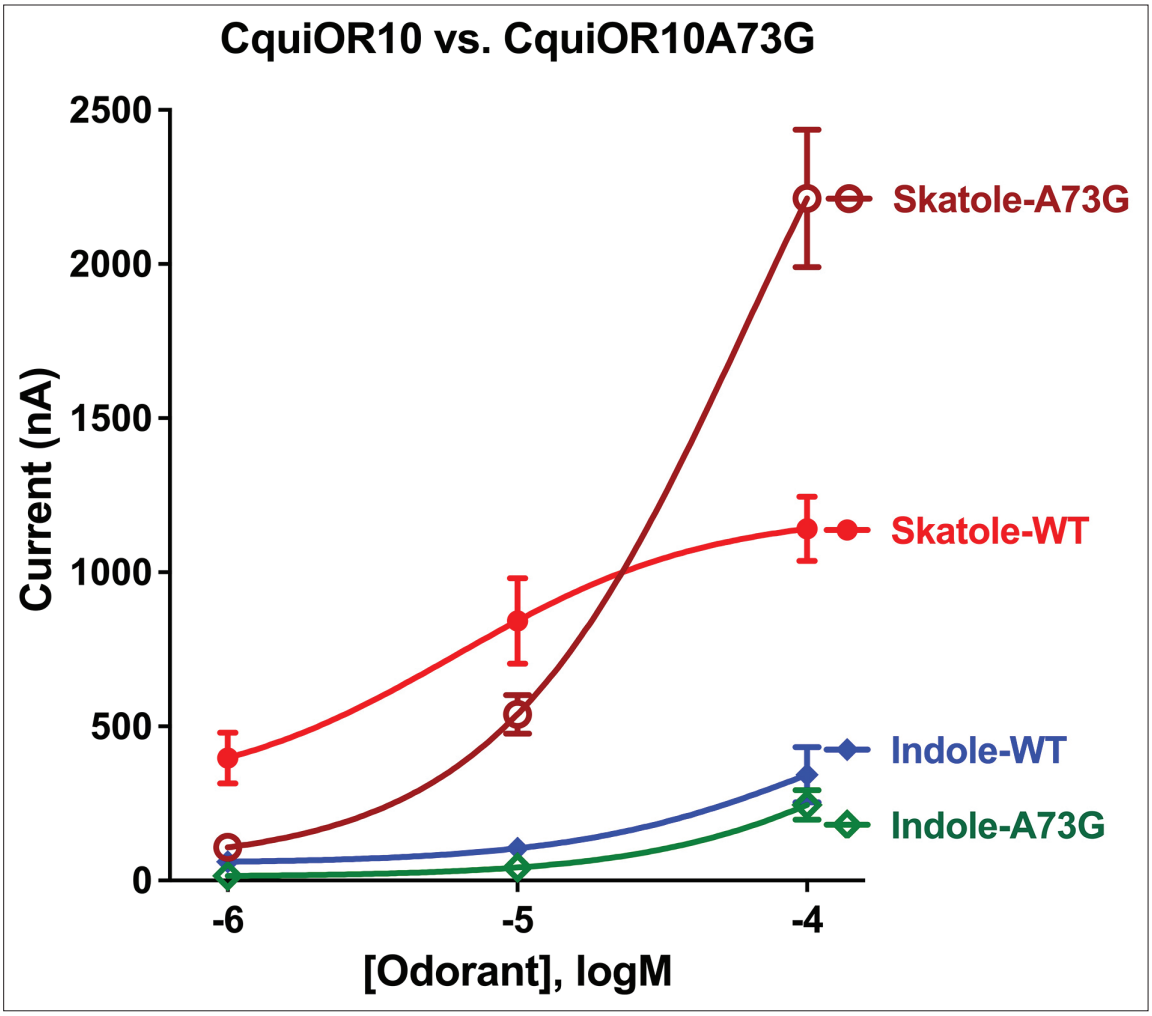

Figure 8—figure supplement 2. Quantification of single-point mutations around A73.

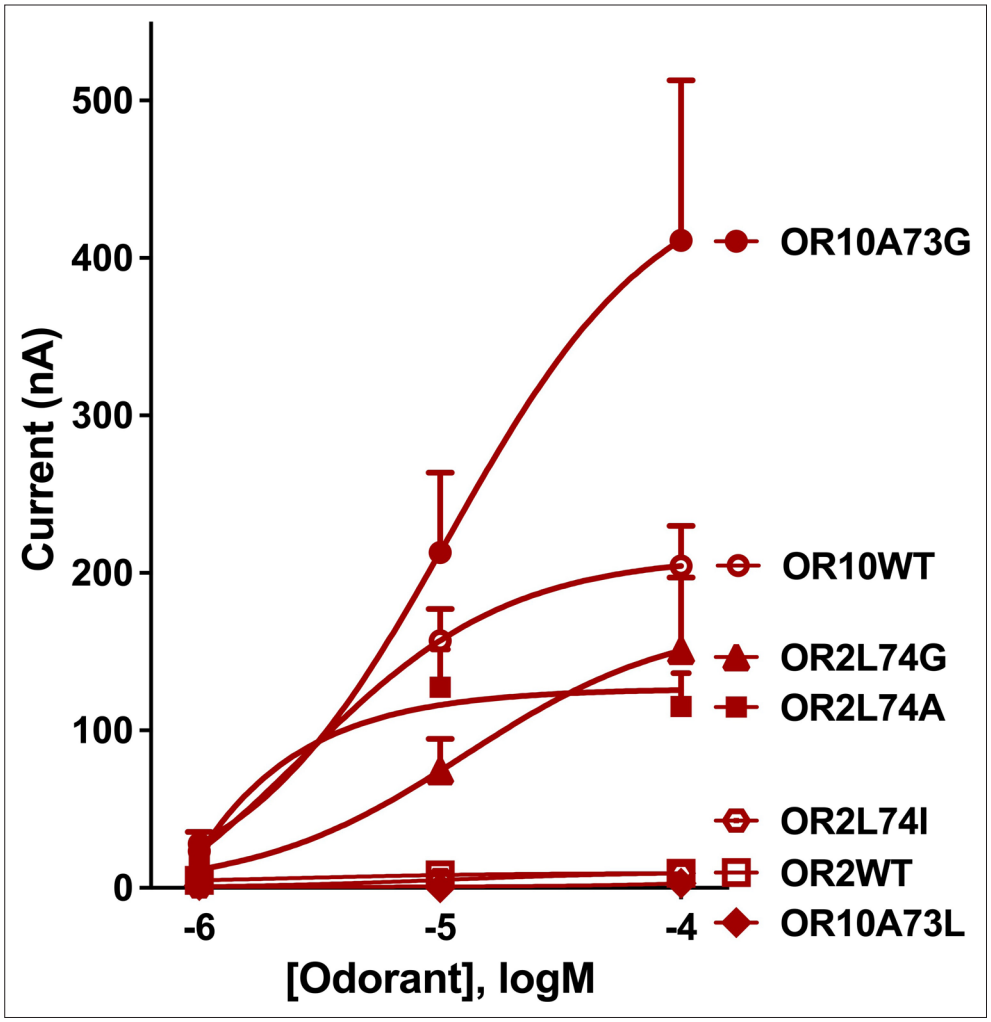

Figure 9. Concentration-dependent responses elicited by 3-ethylindole in oocytes co-expressing CquiOrco with CquiOR10, CquiOR2, or single-point mutants. Bars represent SEM (n = 4–10).

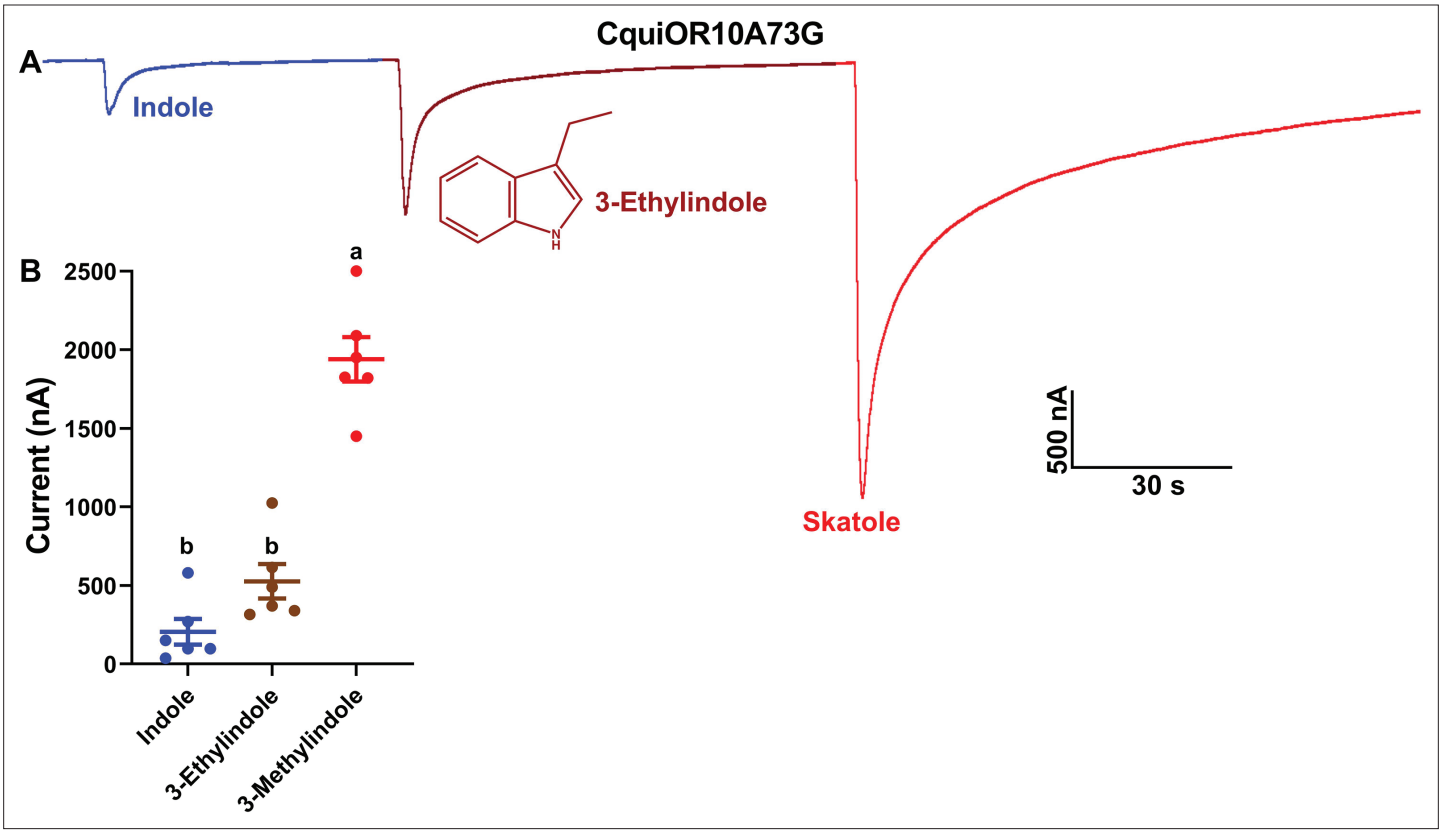

Figure 9—figure supplement 1. Representative trace of the responses of CquiOR10A73G/CquiOrco-expressing oocyte to indole, skatole, and 3-ethylindole (brown). (**A**) Representative trace recorded after challenging an oocyte with the three odorants at the same dose (100 μM). (**B**) Quantification of responses from six different oocytes. Columns with the same letter are not significantly different (Repeated measures, one-way ANOVA).