

## Supplementary Data

### **BIOENGINEERING ANABOLIC VITAMIN D-25-HYDROXYLASE ACTIVITY INTO THE HUMAN VITAMIN D CATABOLIC ENZYME, CYTOCHROME P450 CYP24A1, BY A V391L MUTATION**

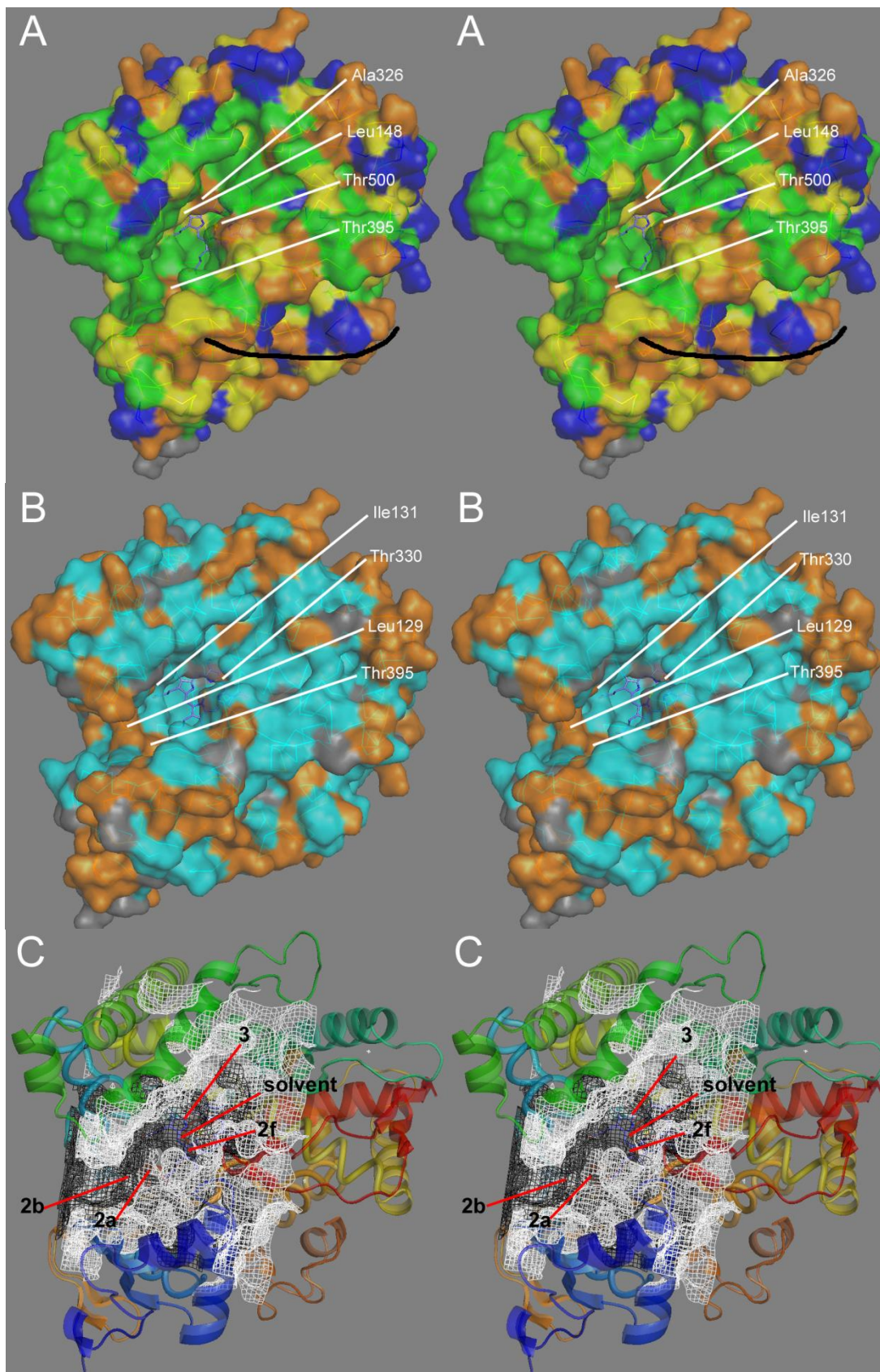
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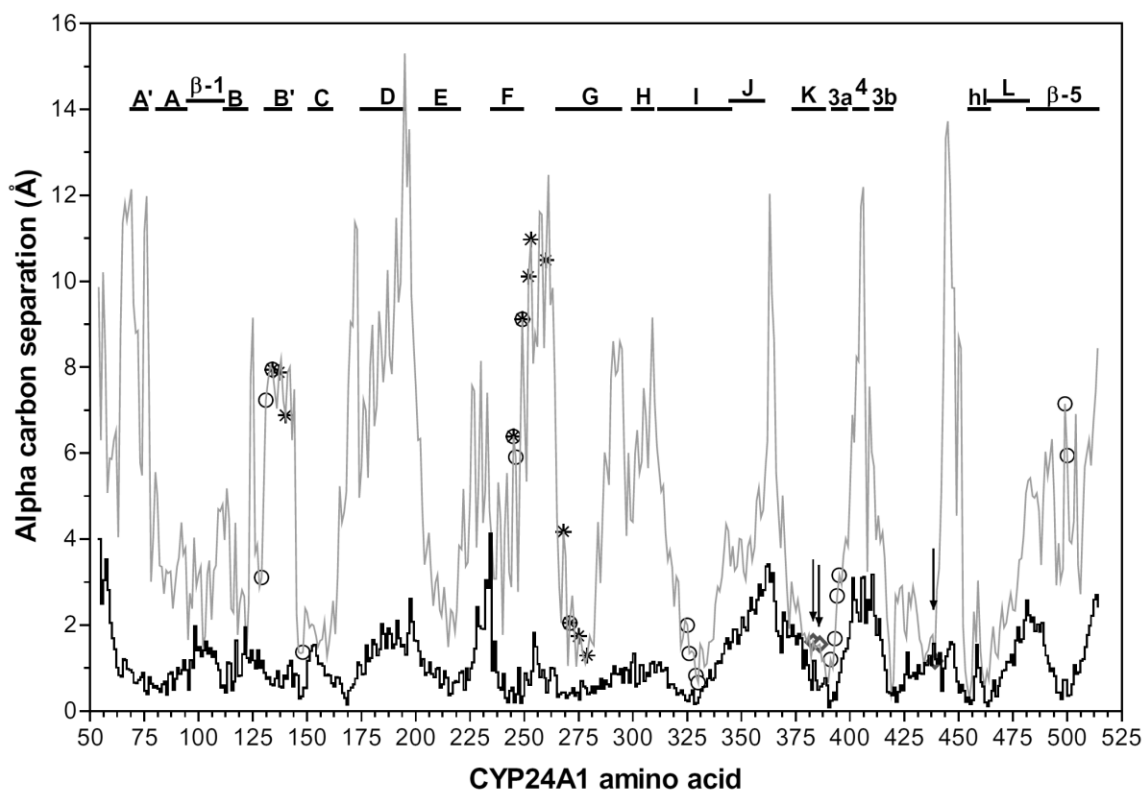
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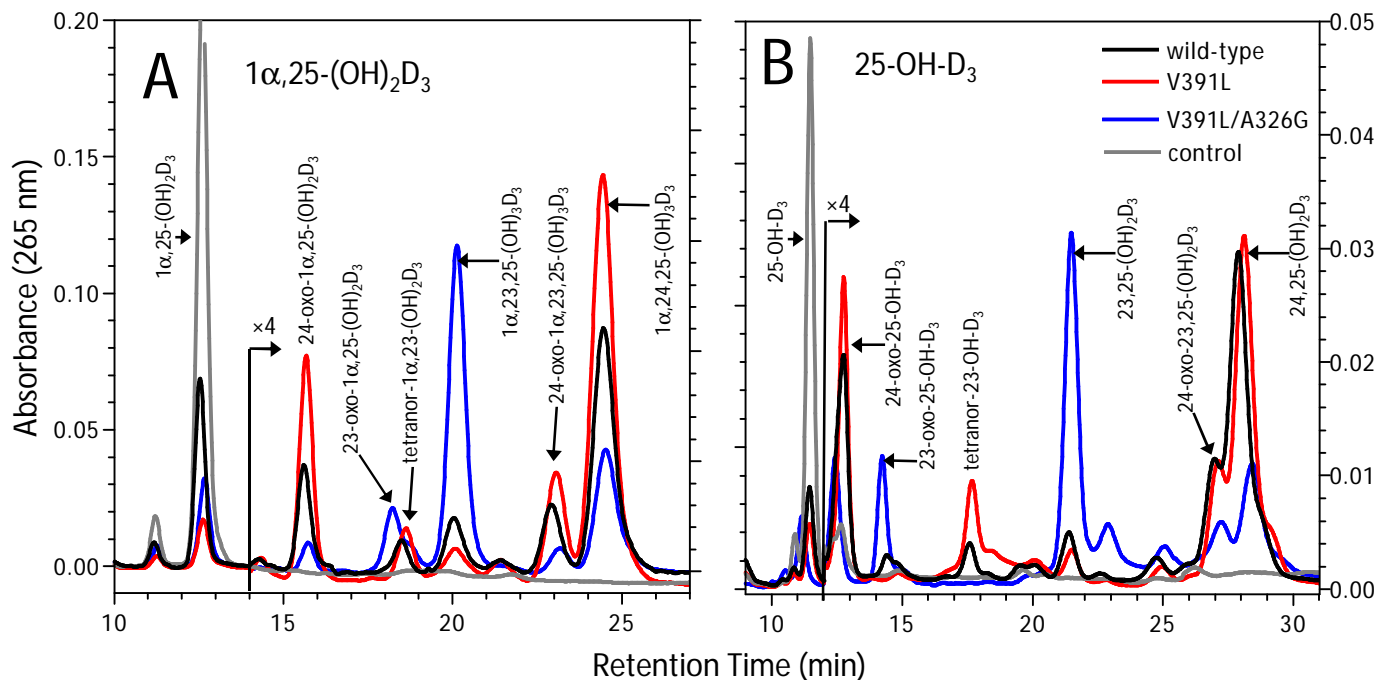
**Fig. S1.** Stereograms of the rCYP24A1 crystal structure. Panel A shows the degree of conserved residues in 53 species orthologs, >95% green, 80-95% yellow, 60-80% orange, <60% blue. Panel B shows amino acid rotamer variation in two crystal structures (two chains each) with invariant rotamers (cyan), two or more rotamers (orange), and nonrotameric alanine, glycine, and proline (gray). Panel C shows the hCYP24A1 model active site cavity within the rCYP24A1 crystal structure cavity and the orientation of various access channel trajectories: pw2a, pw2b, pw2f, pw3, and solvent channel. Evidence suggests that pw2a operates in CYP24A1.



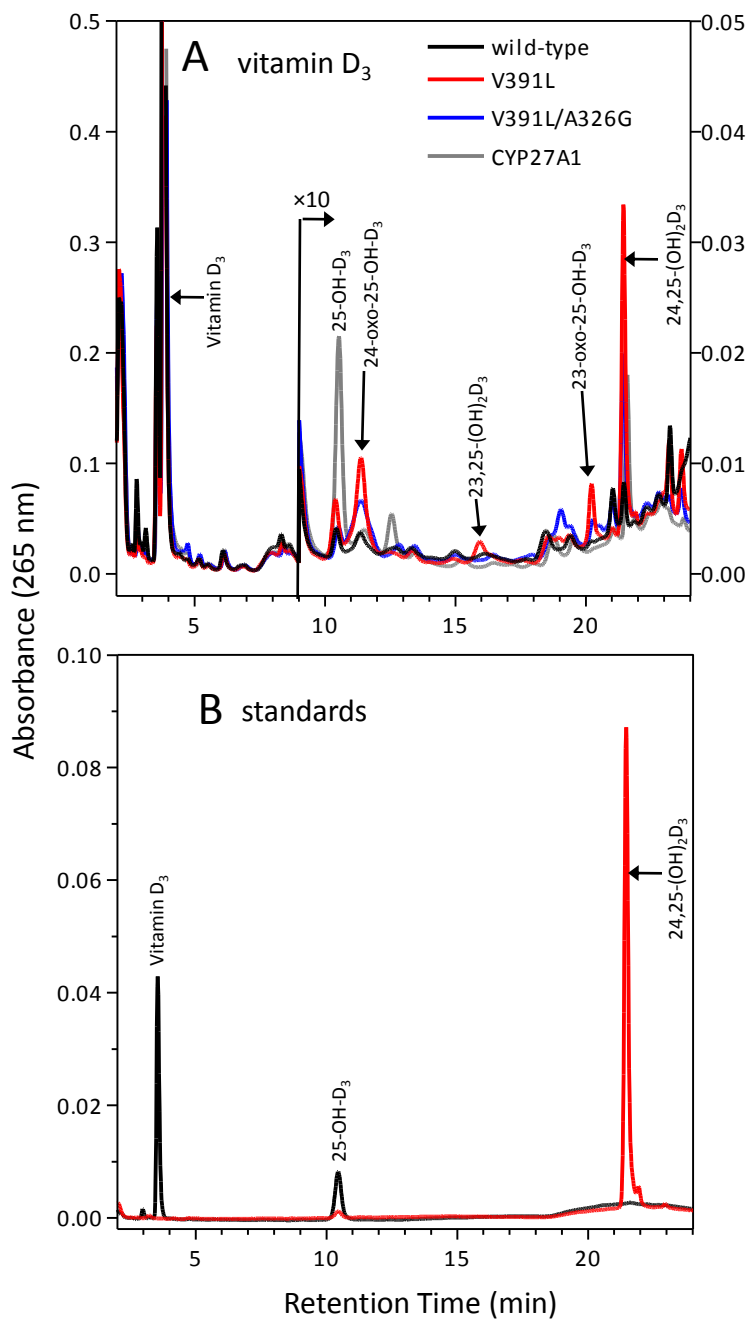
**Fig. S2.** Alpha carbon separation between rCYP24A1 structures (CHAPS chain A and Cymal-5 chain A; dark line) and CHAPS chain A and a model of hCYP24A1 (grey line). Positions of aromatic cluster residues (\*) and active site contact residues (o) and the ERR-triad residues (arrows) are indicated. The average RMSD for CHAPS-Cymal-5 and CHAPS-model were 1.33 Å and 5.56 Å.

	I-helix (OBS)			⇒	K-helix ERR triad		Beta-3a	
	322	326	330		383	386	391	396
CYP24A1.human	: SKKELYAAVTE	LQIAAVETT	TANSLMWILYNLSRN		MPYLKACLKES	MRLT	PSVPFTT	RTRL
Anole	: SKKELYAAIT	LQIAGVETT	TANSLWALYNI	SCN	MPYLKACLKES	MRLT	PSVPFTT	RTRL
Baboon	: SKKELYAAVTE	LQIAAVETT	TANSLMWILYNLSRN		MPYLKACLKES	MRLT	PSVPFTT	RTRL
Bat.Mega	: SKKELYAAVTE	LQIAAVETT	TANSLMWLLYNLSRN		MPYLKACLKES	MRLT	PSVPFTT	RTRL
Bat.Micro	: SKKELYAAVTE	LQIAAVETT	TANSLWLLYNLSRN		MPYLKACLKES	MRLT	PSVPFTT	RTRL
Bovine	: SKKELYAAVTE	LQIAAVETT	TANSLMWILYNLSRN		MPYLKACLKES	MRLN	PTVPFTT	RTRL
Bushbaby	: SKKELYGAVTE	LQGGVETT	TANSLMWILYNLSRN		MPYLKACLKES	MRLT	PSVPFTS	RTRL
Chicken	: SKKELYATIAE	LQIAGVETT	TANSLWALYNI	SRN	MPYLKACLKES	MRLT	PSVPFTT	RTRI
Chimpanzie	: SKKELYAAVTE	LQIAAVETT	TANSLMWILYNLSRN		MPYLKACLKES	MRLT	PSIPFTT	RTRL
Dog	: SKKELYAAVTE	LQIAAVETT	TANSLMWILYNLSRN		MPYLKACLKES	MRLT	PSVPFTT	RTRL
Dolphin	: SKKELYAAVTE	LQIAAVETT	TANSLMWILFNLSRN		MPYLKACLKES	MRLT	PSVPFTT	RTRL
Duck	: SRKELYAAIT	LQIAGVETT	TANSLWALYNI	SRN	MPYLKACLKES	MRLT	PSVPFTT	RTRI
Finch.Zebra	: SRKELYAAIT	LQIAGVETT	TANSLWALYNI	SRN	MPYLKACLKES	MRLT	PSVPFTT	RTRI
Guineapig	: SKKELYAAIT	LQIAGVETT	TANSLMWILYNLSRN		MPYLKACLKES	MRLT	PSVPFTT	RTRL
Hedgehog	: SKKELYAAVTE	LQIAAVETT	TANSLMWILYNLSRN		MPYLKACMKES	MRLT	PSVPFTT	RTRL
Horse	: SKKELYAAVTE	LQIAAVETT	TANSLMWLLYNLSRN		MPYLKACLKES	MRLT	PSVPFTT	RTRL
Hedgehog.Lesser	: SKKELYAAVTE	LQIAAVETT	TANSLMWLLYNLSRN		MPYLKACL	ESMRLT	PSVPFTT	RTRL
Macaque	: SKKELYAAVTE	LQIAAVETT	TANSLMWILYNLSRN		MPYLKACLKES	MRLT	PSVPFTT	RTRL
Opossum.S.Am.	: SKKELYAAVTE	LQIAGVETT	TANSLWVLYNLSRN		LPYLKACLKES	MRLT	PSVPFTT	RTRL
Opossum.Brushtail	: SKKELYAAVTE	LQIAGVETT	TANSLWVLYNLSRN		MPYLKASLKES	MRLT	PSVPFTT	RTRL
Opossum.N.Am.	: SKKELYAAVTE	LQIAGVETT	TANSLWVLYNLSRN		LPYLKACLKES	MRLT	PSVPFTT	RTRL
Marmoset	: SKKELYAAVTE	LQIAAVETT	TANSLMWILYNLSRN		MPYLKACLKES	MRLT	PSVPFTT	RTRL
Mouse	: SKKELYAAVTE	LQIAAVETT	TANSLMWILYNLSRN		MPYLKACLKES	MRLT	PSVPFTT	RTRL
MouseLemur	: SKKELYAAVTE	LQIAGVETT	TANSLMWILYNLSRN		MPYLKACLKES	MRLT	PSVPFTT	RTRL
Orangutan	: SKKELYAAVTE	LQIAAVETT	TANSLMWILYNLSRN		MPYLKACLKES	MRLT	PSVPFTT	RTRL
Pig	: SKKELYASVTE	LQIAAIETT	TANSLMWILYNLSRN		MPYLKACLKES	MRLT	PSVPFTT	RTRL
Platypus	: SKKELYATVTE	LQIAAVETT	TANSLMWILYNLSRN		LPYLKACLKES	MRLT	PSVPFTT	RTRL
Rabbit	: SKKELYAAVTE	LQIAAVETT	TANSLMWVLYNLSRN		LPYLKACLKES	MRLT	PTVPFTT	RTRL
Rat	: SKKELYAAVTE	LQIAAVETT	TANSLMWILYNLSRN		MPYLKACLKES	MRLT	PSVPFTT	RTRL
Tarsier	: SKKELYAAVTE	LQIAAVETT	TANSLMWILYNLSRN		MPYLKACLKES	MRLT	PSVPFTT	RTRL
Treeshrew	: SKKELYAAVTE	LQIAAVETT	TANSLMWILYNLSRN		MPYLKACLKES	MRLT	PSVPFTT	RTRL
Turkey	: SKKELYATIAE	LQIAGVETT	TANSLWALYNI	SRN	MPYLKACLKES	MRLT	PSVPFTT	RTRI
Wallaby	: SKKELYAAVTE	LQIAGVETT	TANSLWVLYNLSRN		MPYLKACLKES	MRLT	PSVPFTT	RTRL
Fish.Danio	: TKKELYAATTE	LQVGGVETT	TANSLWVIFNLSRN		MPYLKACLKES	MRLS	PSVPFTS	RTRL
Fish.FW.Puffer	: SKKELYAAIT	LQIGGVETT	TANSLWVIFNLSRN		MPYLKACLKES	MRLS	PSVPFTS	RTRL
Fish.Lamprey	: TKKELYAATTE	LQVGGVETT	TANSLWVIFNLSRN		MPYLKACLKES	MRLS	PSVPFTS	RTRL
Fish.Medaka	: SKKELYAAIT	LQVGGVETT	TANSLWVIFNLSRN		MPYLKACLKES	MRLS	PSVPFTS	RTRL
Fish.Mudsucker	: SKKELYAAIT	LQIGGVETT	TANSLWVIFNLSRN		MPFLKACLKES	MRLS	PSVPFTS	RTRL
Fish.Stickleback	: SKKELYAAIT	LQIGGVETT	TANSLWVIFNLSRN		MPYLKACLKES	MRLS	PSVPFTS	RTRL
Fish.Fugu	: SKKELYAAIT	LQIGGVETT	TANSLWVIFNLSRN		MPYLKACLKES	MRLS	PSVPFTS	RTRL
Frog	: SKKEMYATITE	MLIGAVETT	TANSLWVIFNLSRN		MPYLKACLKES	MRLT	PSIPFTT	RTRL

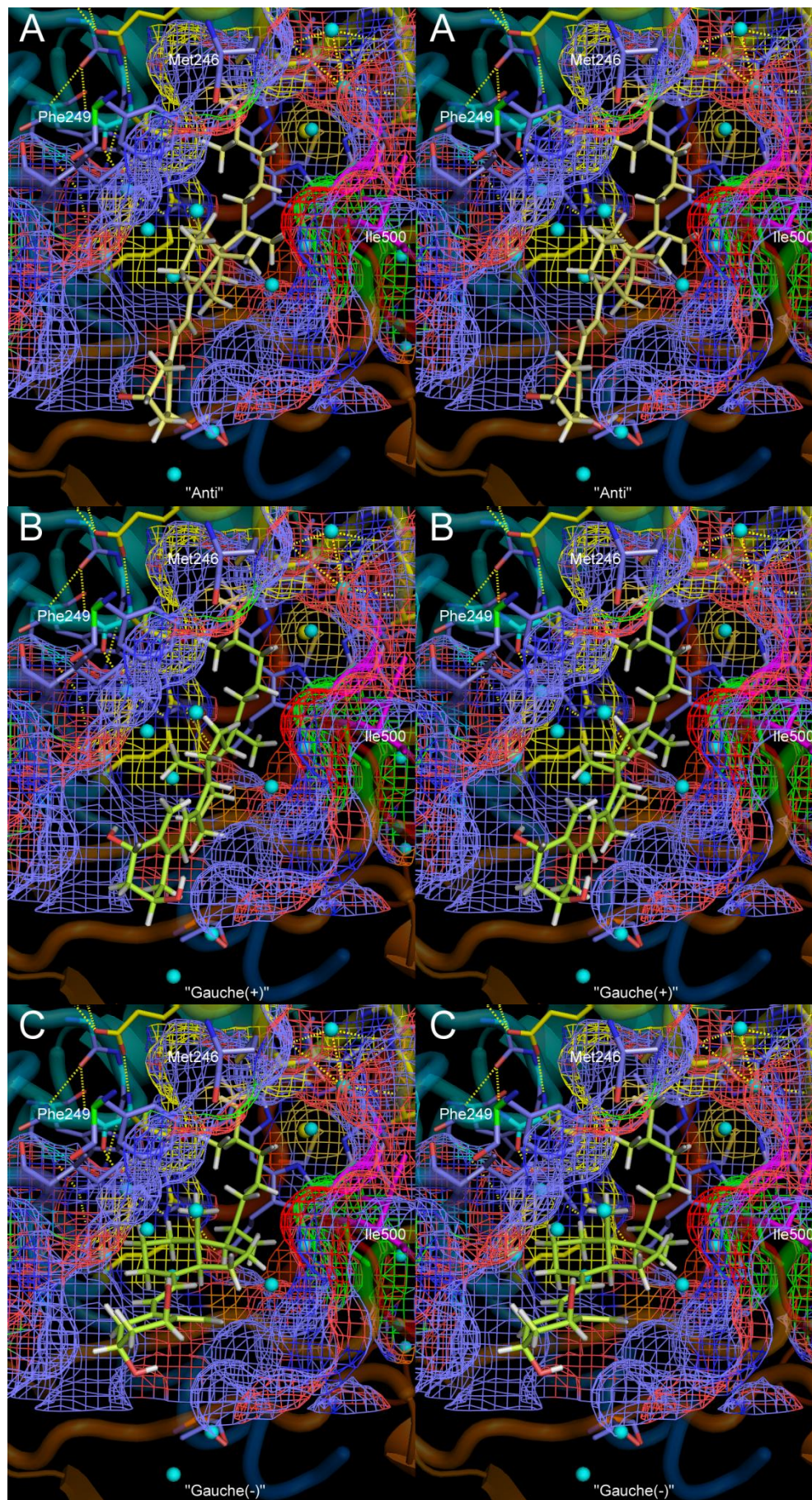
**Fig. S3.** Partial CYP24A1 sequence alignment of 41 species showing the I- and K-helices and beta-3a strand. Light shaded residues indicate greater than 95% identity. Dark shaded residues indicate the structurally important residues Glu322 (stabilizes contact residue Leu148 in the B/C loop), Ala326 (position regulating 23- or 24-hydroxylation), Thr330 (stabilizes the Oxygen Binding Site (OBS) in the I-helix), Glu383 and Arg386 (the first two residues in the ERR triad), Val391 (substrate contact residue mutated in this study), and Arg396 (heme A-ring propionate binding residue).



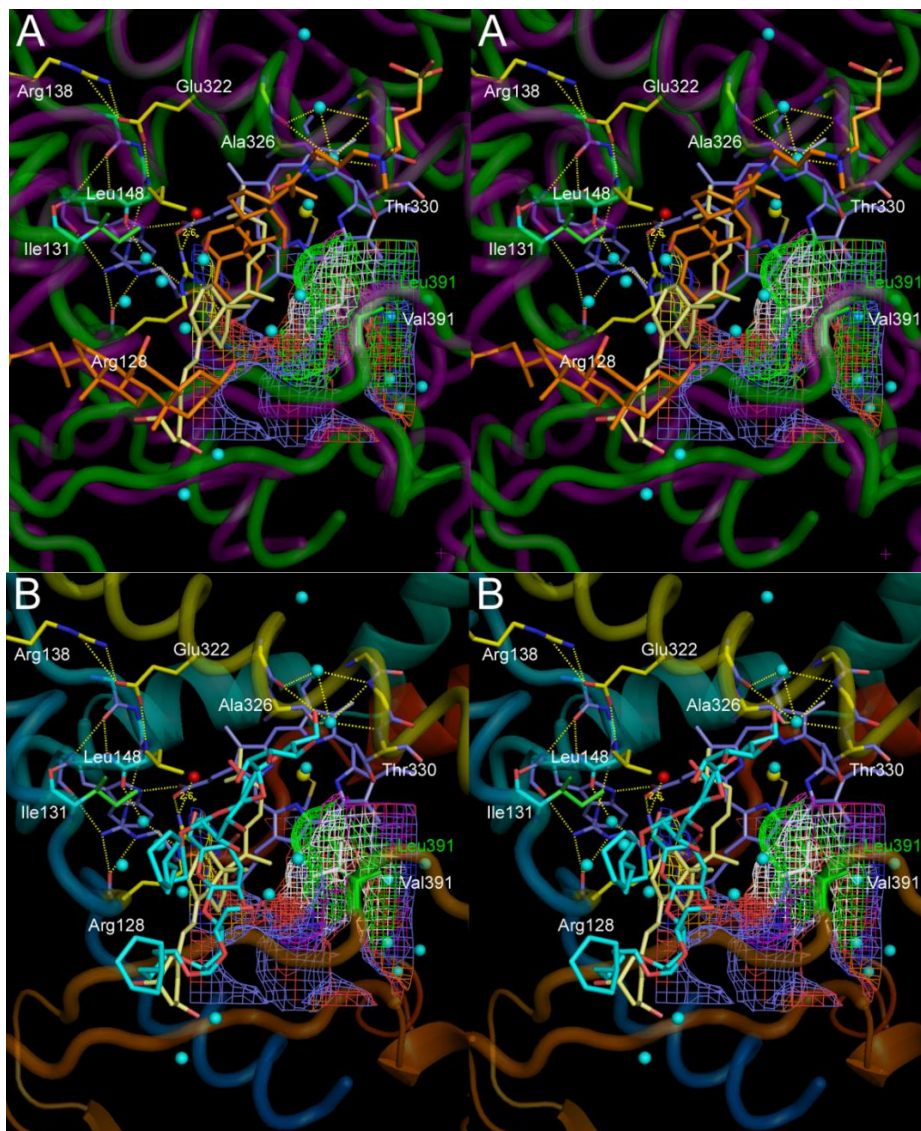
**Fig. S4.** Metabolism of 25-OH-D<sub>3</sub> by V391L-modified CYP24A1. HPLC profiles of the metabolism of 1 $\alpha$ ,25-(OH)<sub>2</sub>D<sub>3</sub> (A) and 25-OH-D<sub>3</sub> (B) by the CYP24A1 mutants under investigation. The legend in panel B, also applies to panel A. The right hand y-axes represent the expanded scale. A quantitative summary of the chromatographic data shown in this figures is presented in Table 1.



**Fig. S5.** Metabolism of vitamin D<sub>3</sub> by V391L-modified CYP24A1. HPLC profiles of the metabolism of vitamin D<sub>3</sub> by mutant CYP24A1 and wild-type CYP27A1(A). HPLC analysis of synthetic standards is shown in panel B. The right hand y-axis in A represents the expanded scale.

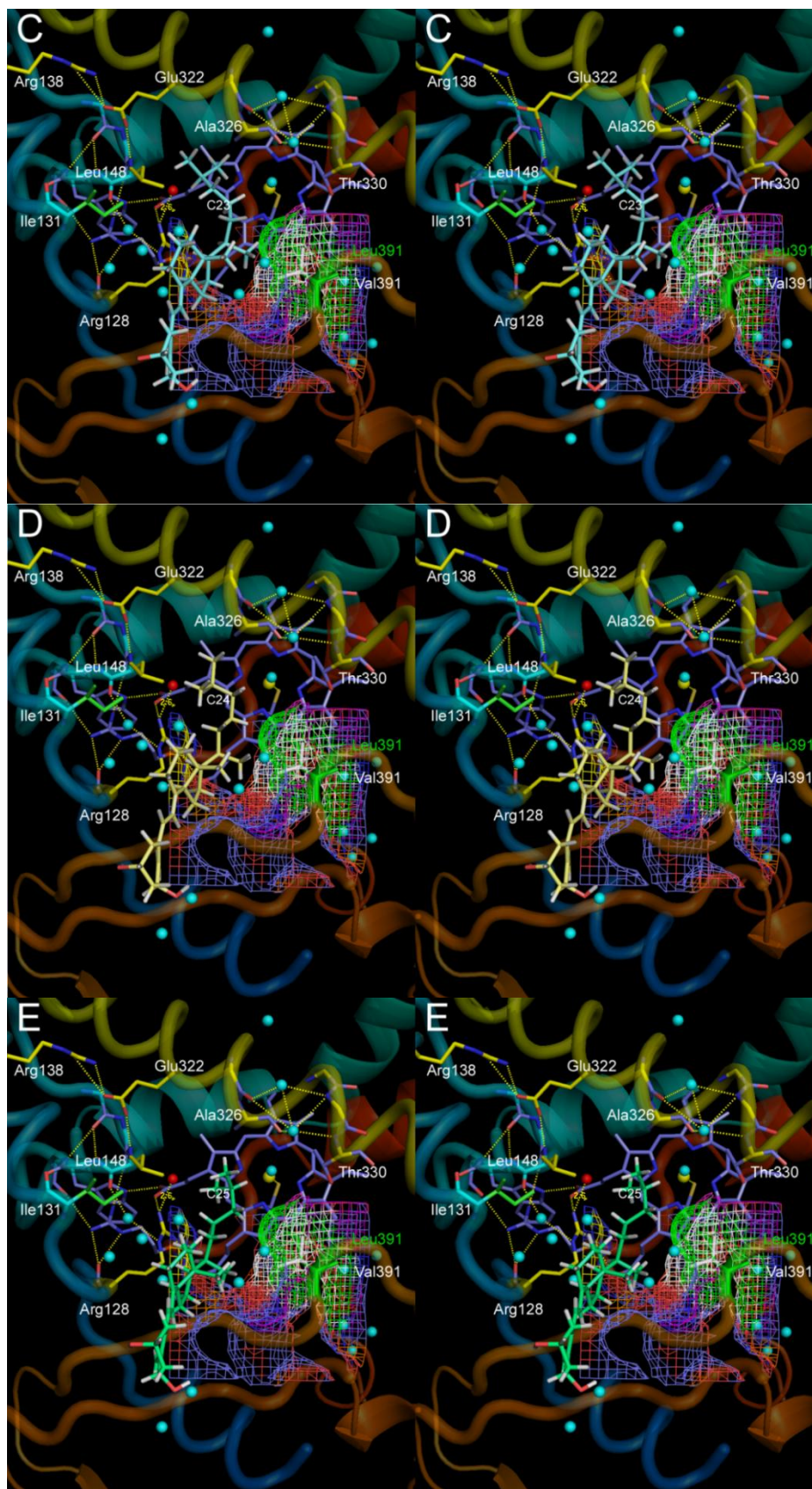


**Fig. S6.** The docking of the “anti”, gauche(+), and gauche(-) conformations of the  $1\alpha$ -OH- $D_3$  side chain (yellow or green) in the active site cavity of rCYP24A1. These panels illustrate that different conformations of the vitamin D side chain are easily accommodated in the expansive active site cavity of the rCYP24A1 crystal structure. Water molecules are shown as blue spheres.

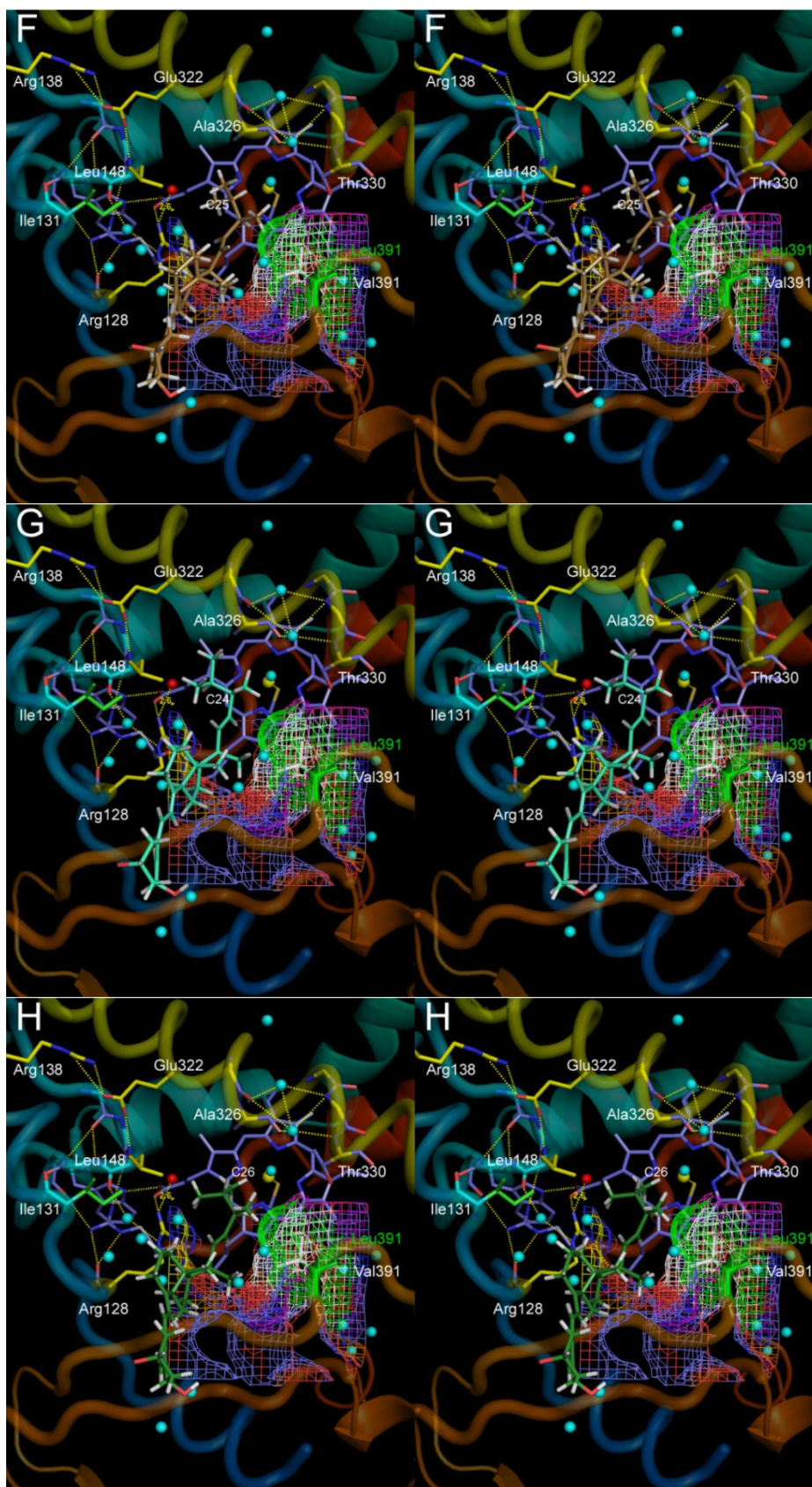


**Fig. S7 A-B.** Stereograms of regiospecifically docked vitamin D structures. Panel A shows the alignment of rCYP24A1 crystal structure (green) with our model of hCYP24A1 (purple) with  $1\alpha$ -OH-D<sub>3</sub> docked for 24-hydroxylation (yellow). The  $1\alpha$ -OH-D<sub>3</sub> is seen to partially overlap with two of the CHAPS surfactant molecules from rCYP24A1 chain A (3k9v.pdb, orange) which occupy non-reactive orientations in the active site. Panel B shows even less overlap with the two Cymol-5 surfactant molecules from rCYP24A1 chain A (3k9y.pdb, cyan) which appear to be oriented in a pw2a access channel trajectory. Water molecules are shown as blue spheres. This figure continues on page S-8.





**Fig. S7 C-E.** Panels C, D, and E allow comparison of  $1\alpha$ -OH- $D_3$  oriented for 23-, 24-, and 25-hydroxylation, respectively.  $1\alpha$ -OH- $D_3$  is color-coded for docked orientation: cyan for 23-hydroxylation (C), yellow for 24-hydroxylation (D) and lime green for 25-hydroxylation (E). In each case, there appears to be less conflict between C21 and with the wild-type Val391 (white) and mutant Leu391 (green) surfaces which is consistent with the increased activity of the V391L mutants. Key functional and substrate contact residues are labeled (the rCYP24A1 residue Met148 is replaced by the human ortholog residue Leu148) and the position of a hypothetical water molecule capable of bridging the heme-bound Arg128 to a substrate 25-hydroxyl group is shown as a red sphere near Leu148. All other water molecules are shown as blue spheres. This figure continues on page S-9.



**Fig. S7 F-H.** Panels F, G and H show  $1\alpha$ -OH- $D_2$  oriented for 25-, 24-, and 26-hydroxylation respectively.  $1\alpha$ -OH- $D_2$  is color-coded for docked orientation: brown for 25-hydroxylation (F), cyan for 24-hydroxylation (G) and green for 26-hydroxylation (H). The steric contact of the 24S-methyl group with the Leu391 surface in panel F is consistent with the reduced 25-hydroxylation seen with the V391L, and consistent with the observation of being a better 24- and 26-hydroxylase of  $1\alpha$ -OH- $D_2$ . Water molecules are shown as blue spheres.