<u>Supplemental Information for</u>:

Dock & Design: Engineering specificity for alternative pimaradiene outcome with the *ent*-kaurene synthase from *Bradyrhizobium japonicum*

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Figure S1: Constraints imposed for investigation of F72Y variant.

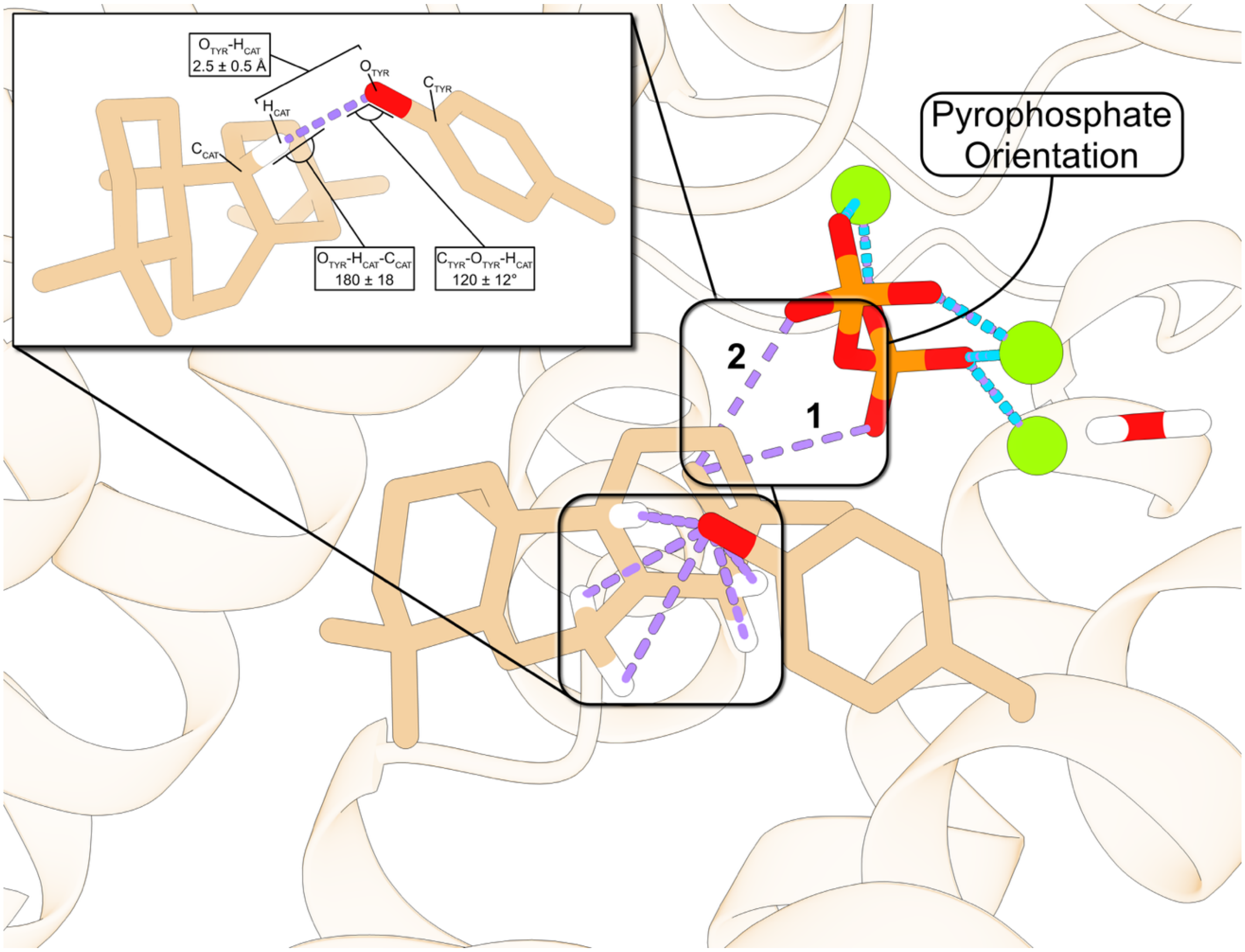

Table S1: Docking results for production of **5** by BjKS:F72Y using introduced hydroxyl as catalytic base to remove the proton from C9 in **A** or, following a 1,2-hydride shift, from C8 in *ent*-pimara-15-en-9-yl$^+$. Conformer libraries were acquired for both the C9-yl and C8-yl intermediates which in the case of the C8-yl$^+$ intermediate had been previously created whereas for the C9-yl intermediate was generated. To generate the C9-yl$^+$ intermediate, an initial structure was optimized using density functional theory using the Gaussian software suite with the B3LYP functional and the 6-31+G(d,p) functional. Conformers were generated from this structure using CREST before being optimized as before and filtered to be within 5 kcal of the lowest energy conformer. Using these conformer libraries, 2,500 docking simulations were performed for each combination of docking orientation (2 active site facing oxygens on pyrophosphate) for each carbocation using the described constraints. The resultant (10,000) structures were combined and filtered to select the energetically most favorable structural poses as described, with the number of poses grouped by pyrophosphate orientation presented here.

F72Y	C8 Orientation 1	C9 Orientation 1
Poses	65	23
	C8 Orientation 2	C9 Orientation 2
Poses	25	16
SUM	90	39

Figure S2: Predicted relative site of deprotonation for production of **5** by BjKS:F72Y.

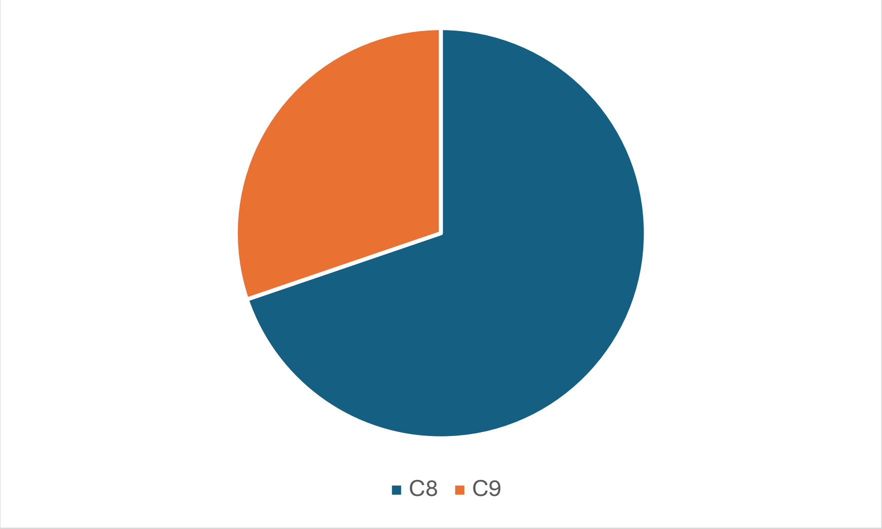

Table S2: Docking results for BjKS:F72Y using introduced hydroxyl to deprotonate **A**. Briefly, using previously generated conformer libraries, 5,000 docking simulations were performed for each combination of docking orientation (2 active site facing oxygens on pyrophosphate) with the 5 protons around the carbocation (C8). The resultant (50,000) structures were combined and filtered to select the energetically most favorable structural poses. The tables present the number of poses passing filtering grouped by pyrophosphate orientation and relevant hydrogen.

F72Y	C7 Orientation 1		C14 Orientation 1		C9 Orientation 1
Deprotonated H	H32	H33	H19	H25	H20
Poses	0	1	0	0	71
	C7 Orientation 2		C14 Orientation 2		C9 Orientation 2
Deprotonated H	H32	H33	H19	H25	H20
Poses	0	2	0	0	19
SUM	3		0		90

Table S3: Residues targeted for *in silico* screen of serine/threonine substitution.

G32S	I166S
L71S	I166T
L71T	V277S
F72S	R281S

Figure S3: Representative diagram of the filtering to select the energetically most favorable structural poses performed for the initial *TerDockin* design towards production of pimaradienes **3** – **5** via deprotonation of **A** by the hydroxyl introduced by serine or threonine substitution for selected active site residues (Table S3).

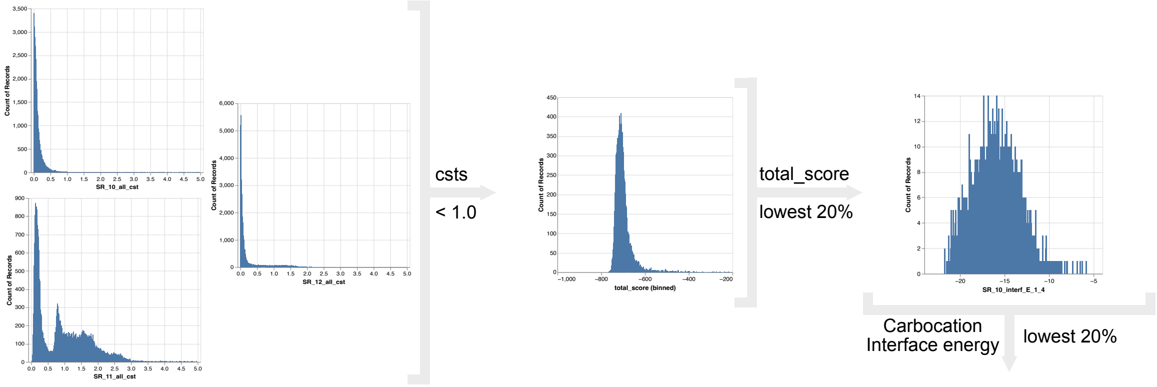

Table S4: Docking results for mutants selected from prospective application of *TerDockin* for production of pimaradienes **3** – **5** via deprotonation of **A** by hydroxyl introduced by serine or threonine substitution. Each (sub)table presents the number of poses passing filtering grouped by pyrophosphate orientation and relevant hydrogen for the indicated variant.

L71S	C7 Orientation 1		C14 Orientation 1		C9 Orientation 1
Deprotonated H	H32	H33	H19	H25	H20
Poses	1	0	122	2	1
	C7 Orientation 2		C14 Orientation 2		C9 Orientation 2
Deprotonated H	H32	H33	H19	H25	H20
Poses	23	0	224	0	1
SUM	24		348		2

I166T	C7 Orientation 1		C14 Orientation 1		C9 Orientation 1
Deprotonated H	H32	H33	H19	H25	H20
Poses	22	150	14	1	0
	C7 Orientation 2		C14 Orientation 2		C9 Orientation 2
Deprotonated H	H32	H33	H19	H25	H20
Poses	3	220	5	0	2
SUM	395		20		2

V277S	C7 Orientation 1		C14 Orientation 1		C9 Orientation 1
Deprotonated H	H32	H33	H19	H25	H20
Poses	0	4	3	0	16
	C7 Orientation 2		C14 Orientation 2		C9 Orientation 2
Deprotonated H	H32	H33	H19	H25	H20
Poses	0	10	0	6	0
SUM	14		9		16

Figure S4: Extracted ion (257+ 272 m/z) chromatograms of indicated mutants.

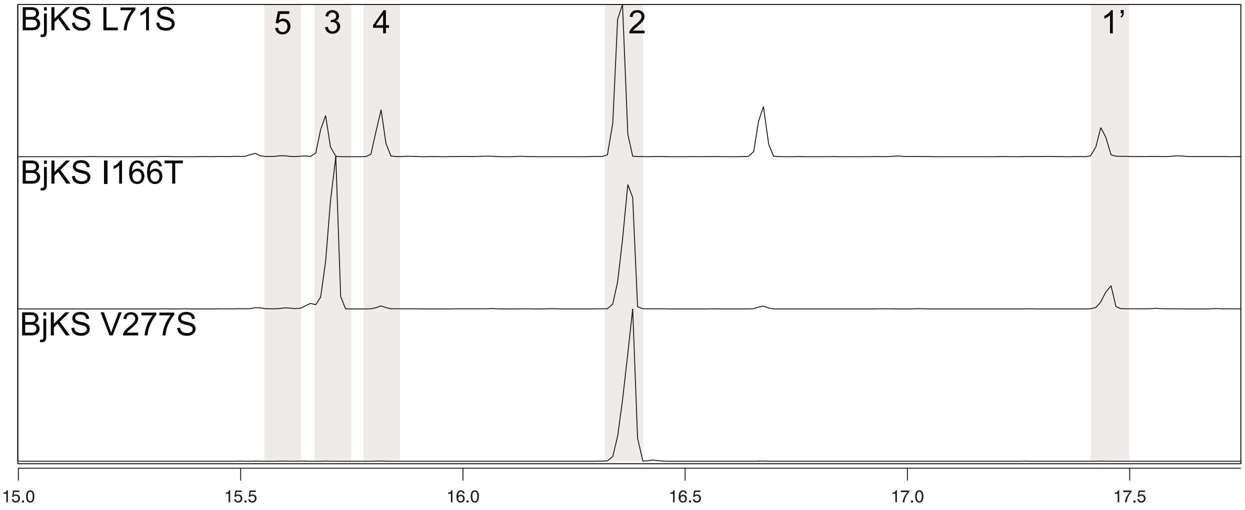

Figure S5: Representative poses for L71S, I166T and V277S, with extended distances indicated.

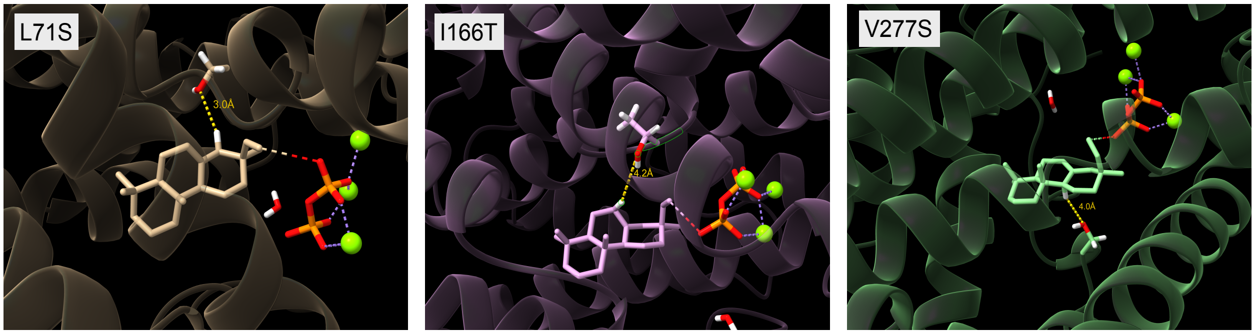

Figure S6: Extracted ion (257+ 272 m/z) chromatograms for A) G32, B) G32T, C) Y280S, and D) Y280T.

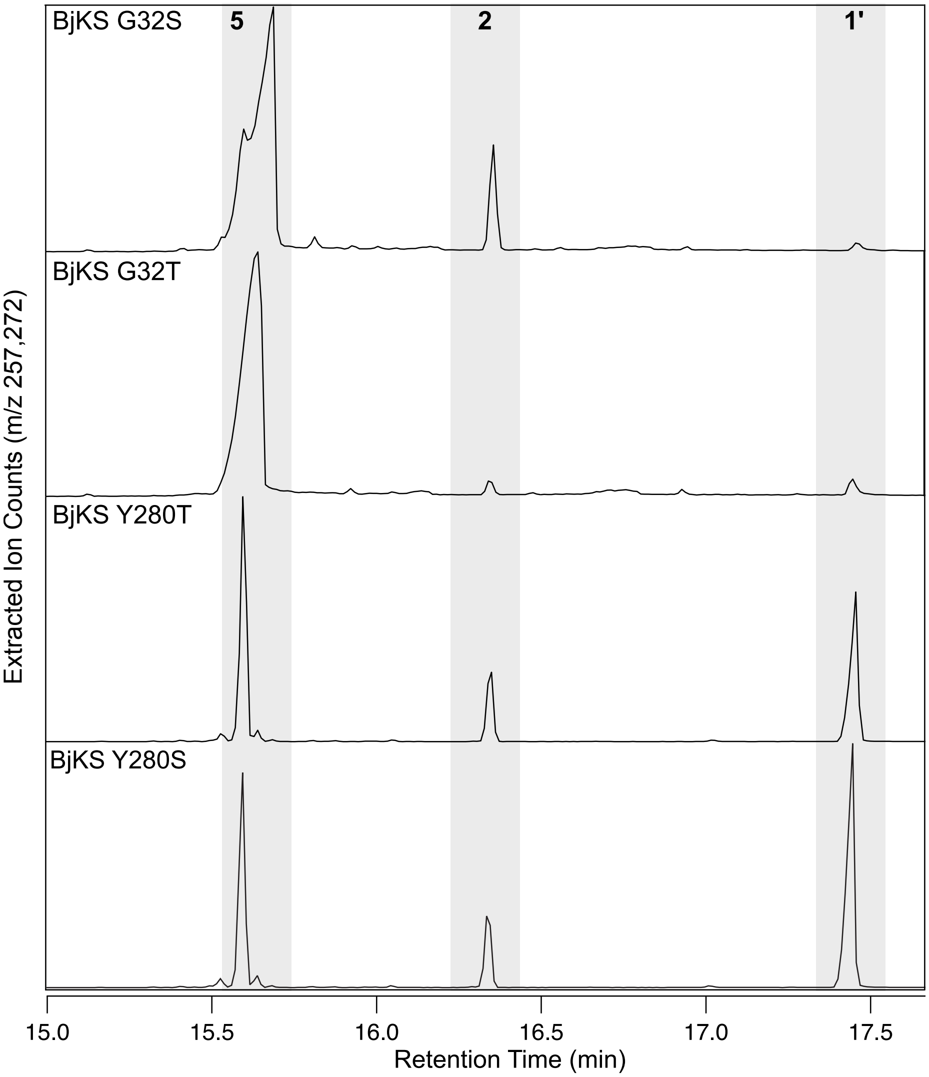

Figure S7: Extracted ion (257+ 272 m/z) chromatogram for BjKS:I166F.

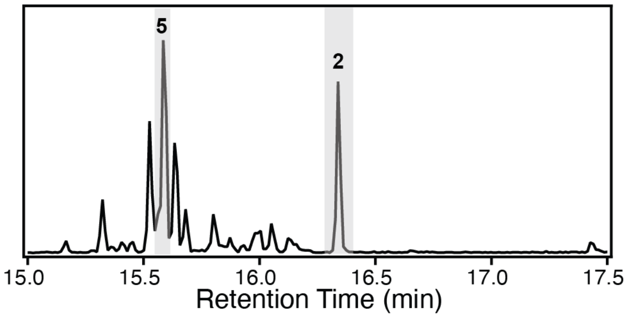

Table S5: BjKS:F72Y/I166F/G32(D,E,H,N,Q,S,T,Y)/Y280X with 5+ Poses Passing Filtering.

mutation	**count**	**mutation2**	**count**	**mutation4**	**count**	**mutation6**	**count**
G32T_280L	17	G32N_280Y	10	**G32S_280D**	**7**	G32E_280N	6
G32N_280T	**15**	G32T_280Y	10	G32T_280G	7	G32D_280S	6
G32N_280F	**15**	G32Q_280C	10	G32H_280L	7	G32Y_280D	6
G32T_280I	15	G32N_280S	10	G32H_280I	7	G32S_280L	6
G32T_280F	15	G32T_280R	10	G32D_280M	7	**G32T_280W**	**5**
G32H_280C	14	**G32D_280F**	**9**	G32E_280T	7	G32N_280R	5
G32H_280F	14	G32H_280R	9	G32Q_280W	7	G32S_280V	5
G32E_280E	13	G32H_280W	9	G32T_280H	7	G32S_280A	5
G32T_280M	13	G32Q_280T	9	G32S_280G	7	G32E_280Y	5
G32H_280Y	13	G32H_280E	9	G32S_280E	7	G32Q_280L	5
G32N_280C	13	G32Q_280M	9	G32Y_280H	7	G32Y_280T	5
G32D_280Y	12	G32H_280T	9	G32Q_280N	7	G32Y_280F	5
G32H_280S	12	G32T_280D	9	G32E_280C	7	G32E_280A	5
G32H_280A	11	G32Q_280R	9	G32Q_280A	7	G32E_280F	5
G32H_280K	11	G32Q_280H	9	G32T_280C	7	G32S_280T	5
G32Q_280F	11	G32N_280L	9	G32N_280D	7	G32S_280Q	5
G32T_280A	11	G32D_280I	9	G32Q_280V	7	G32D_280N	5
G32H_280D	11	G32H_280H	9	G32N_280H	7	G32D_280H	5
G32T_280K	11	**G32H_280V**	**8**	**G32D_280A**	**6**	G32N_280V	5
G32T_280V	11	G32E_280D	8	G32Y_280K	6	G32E_280S	5
G32T_280S	**10**	G32D_280T	8	G32D_280R	6	G32N_280M	5
G32H_280M	10	G32N_280E	8	G32Q_280E	6	G32N_280A	5
G32T_280E	10	G32S_280C	8	G32D_280E	6	G32Y_280S	5
G32Q_280Y	10	G32S_280I	8	G32D_280C	6	G32E_280H	5
G32N_280I	10	G32D_280Q	8	G32E_280M	6	G32S_280Y	5
G32S_280F	10	G32Y_280E	8	G32Q_280G	6	G32N_280K	5
G32H_280Q	10	G32H_280N	8	G32Q_280D	6	G32D_280V	5
G32T_280T	10	G32E_280R	8	G32Q_280S	6	G32S_280W	5

Figure S8: Extracted ion (257+ 272 m/z) chromatograms of indicated mutants.

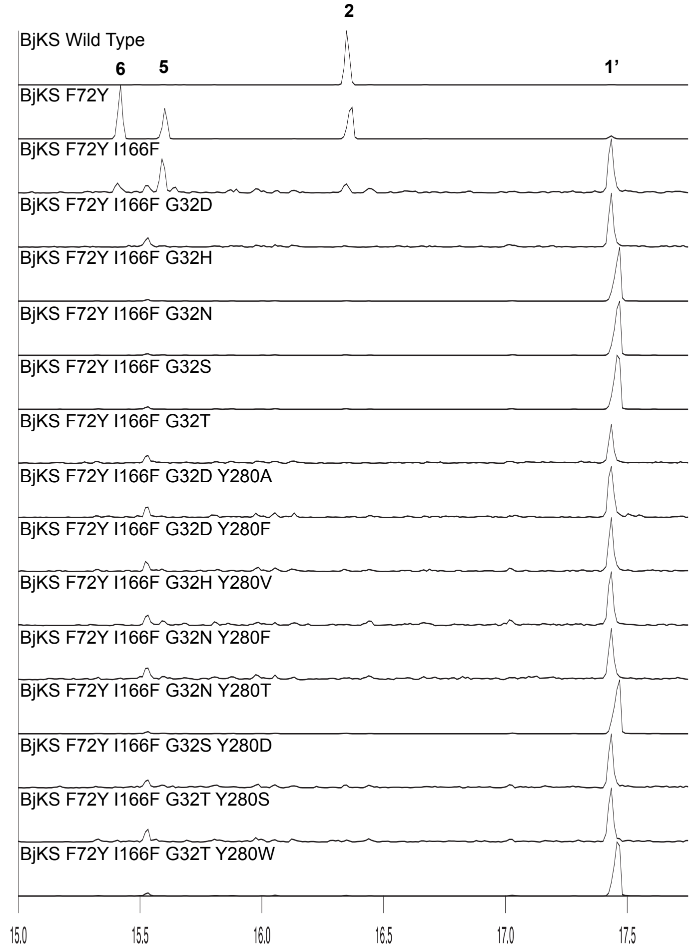

Figure S9: Representative diagram of the filtering performed to select the energetically most favorable variants of F72X and Y280X from *TerDockin* design towards production of **5**.

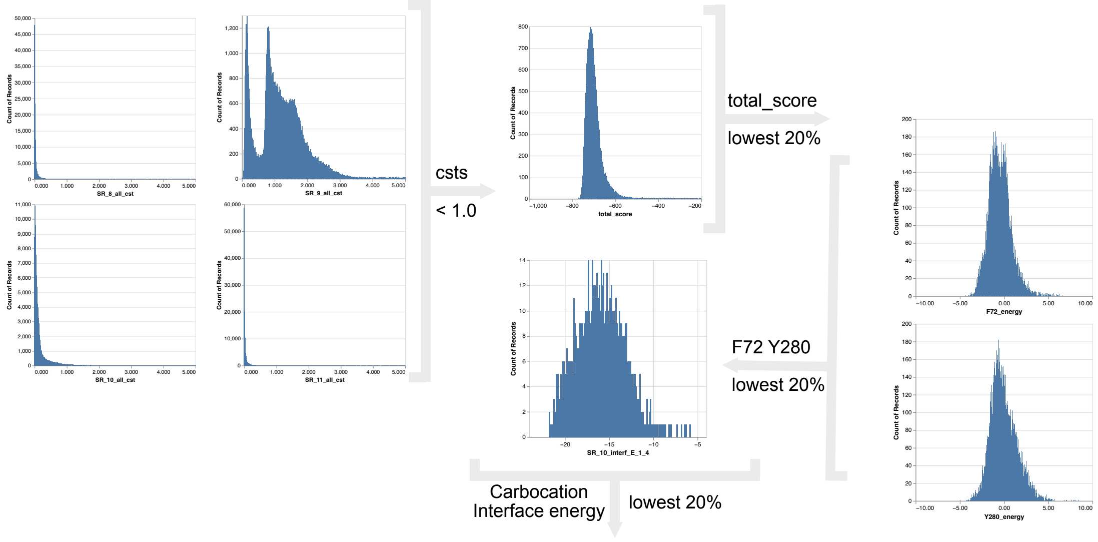

Table S6: BjKS – F72X Y280X with 2+ Poses Passing Filtering.

mut	count	mut2	count2
F72A_Y280T	**6**	**F72W_Y280T**	**2**
F72A_Y280Y	**6**	F72R_Y280D	2
F72V_Y280Y	6	F72Y_Y280D	2
F72H_Y280L	**5**	F72Y_Y280Y	2
F72S_Y280Y	**5**	F72H_Y280V	2
F72H_Y280D	**4**	F72A_Y280S	2
F72H_Y280S	**4**	F72M_Y280Y	2
F72V_Y280D	4	F72N_Y280L	2
F72I_Y280Y	4	F72N_Y280D	2
F72H_Y280Y	4	F72N_Y280Y	2
F72L_Y280H	3	F72Y_Y280H	2
F72A_Y280H	3	F72A_Y280Q	2
F72I_Y280H	3	F72F_Y280Y	2
F72L_Y280T	3	F72V_Y280H	2
F72L_Y280D	3	F72S_Y280F	2
F72H_Y280T	3	F72A_Y280E	2
F72L_Y280Y	3	F72Y_Y280F	2
F72Y_Y280T	3	F72H_Y280F	2
F72D_Y280D	3	F72L_Y280E	2
F72W_Y280D	**2**	F72D_Y280I	2
F72Y_Y280S	**2**	F72S_Y280T	2
F72W_Y280E	**2**	F72Y_Y280L	2

Figure S10: Extracted ion (257+ 272 m/z) chromatograms of indicated mutants.