Supplemental Data

Structure of the Pseudokinase VRK3 Reveals a Degraded Catalytic Site, a Highly Conserved Kinase Fold, and a Putative Regulatory Binding Site

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Table S1. Crystallographic Data and Refinement Statistics

Data Collection	**VRK2**	**VRK3**
Space group	$P2_12_12$	$P2_1$
Cell dimensions [Å and °]	a=70.65, b=157.53, c=56.29	a=91.26, b=54.42, c=91.82, β=91.2
Resolution*	1.7 (1.7-1.8)	2.0 (2.0-2.1)
Unique observations*	67759 (10022)	60969 (8014)
Completeness* [%]	96.9 (93.3)	99.2 (95.4)
Redundancy*	5.8 (5.5)	3.6 (2.6)
R_{merge}*	0.0562 (0.5859)	0.0648 (0.4167)
$I / \sigma I$*	15.3 (2.3)	12.5 (2.2)
Refinement		
Reflections (R_{free} set)	64302 (3413)	57853 (3092)
R_{work} / R_{free}	0.201 / 0.236	0.178 / 0.223
Atoms (P/L/W)#	4828 / 21 / 334	5143 / 20 / 514
B-factors (P/L/W)# [Å^2]	34.1 / 50.0 / 38.5	34.9 / 49.0 / 40.8
RMSD bonds [Å] RMSD [°]	0.015 1.483	0.011 1.265
Ramachandran favorable [%] allowed [%]	 99.6 0.4	 100.0 0.0

Table S2. Superpositions between VRK2 and VRK3 and Other Representative Protein Kinases, as Aligned by DaliLite (Holm and Park, 2000)

Structure (PDB ID: Chain)	**VRK3** (2JII:B)	**VRK2** (2V62:A)	**CK1** (1CSN:A)	**PKA** (1CDK:A)
VRK2 (2V62:A)	2.3 (291,38,35.4)			
CK1 (1CSN:A)	2.1 (271,23,30.3)	2.4 (266,27,29.2)		
PKA (1CDK:A)	3.1 (257,16,22.2)	3.1 (241,18,20)	2.8 (258,19,23.9)	
Sky1p (1Q97:A)	3.0 (272,15,23.6)	3.1 (262,19,21)	2.6 (250,20,22.9)	2.7 (251,26,24)

RMSD is given in Å, followed by additional information on the structural comparison in parentheses: number of aligned positions, % sequence identity, and DaliLite Z-score. Comparisons between VRK3 and VRK2 and paralogs are highlighted in gray.

Table S3. Inhibitor Screening Results for VRK1

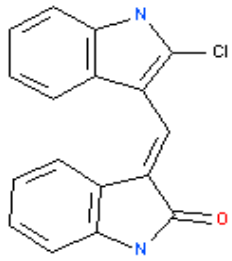
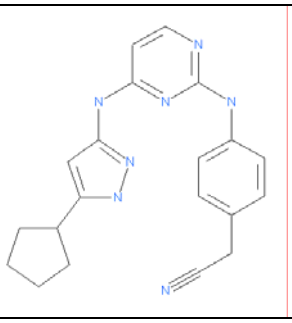
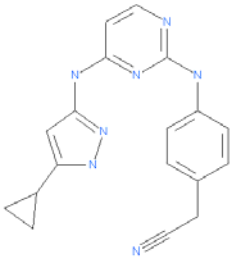
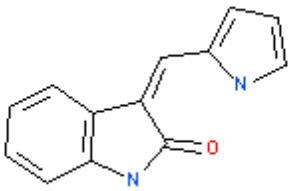
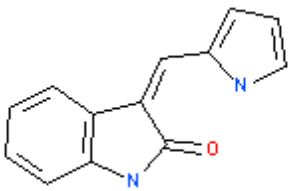
Compound name	Chemical structure	VRK1 Tm Shift [°C]
3-(2-Chloro-3-indolylmethylene)-1,3-dihydroindol-2-one		4
ASC67		4.3
ASC24		4.5
Oxindole I; 3-(1H-Pyrrol-2-ylmethylene)-1,3-dihydroindol-2-one		3.5
5-Iodo-3-[(3,5-dibromo-4-hydroxyphenyl)methylene]-2-indolinone		3.5

Table S4. Inhibitor Screening Results for VRK2

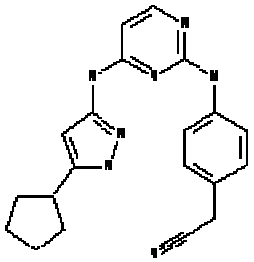
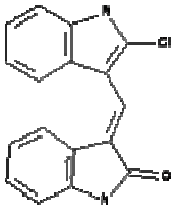
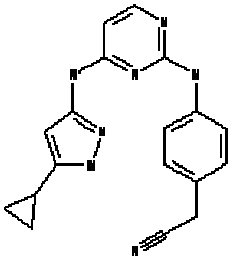
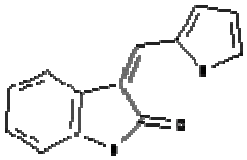
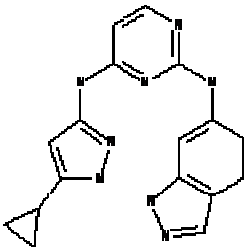
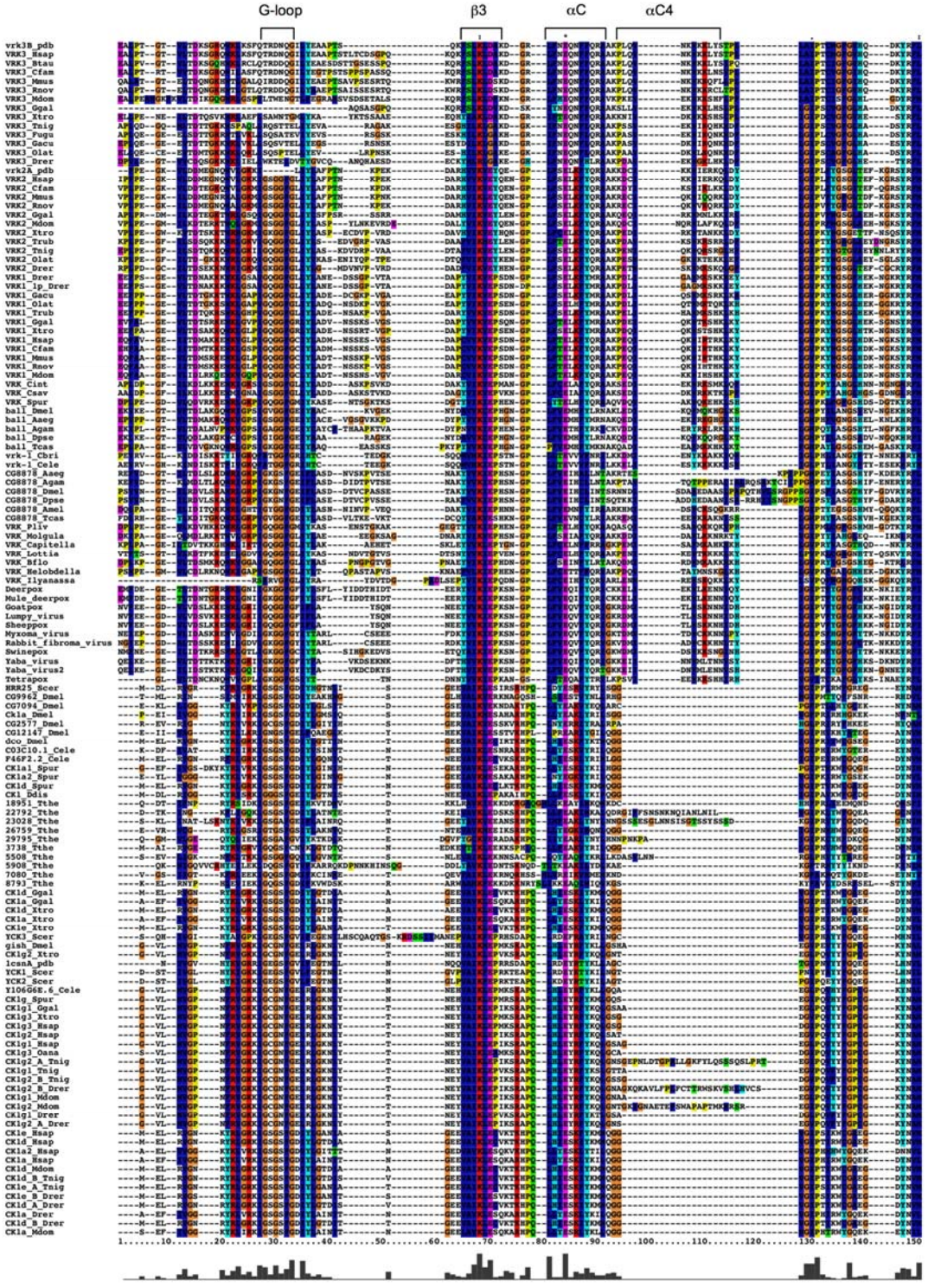
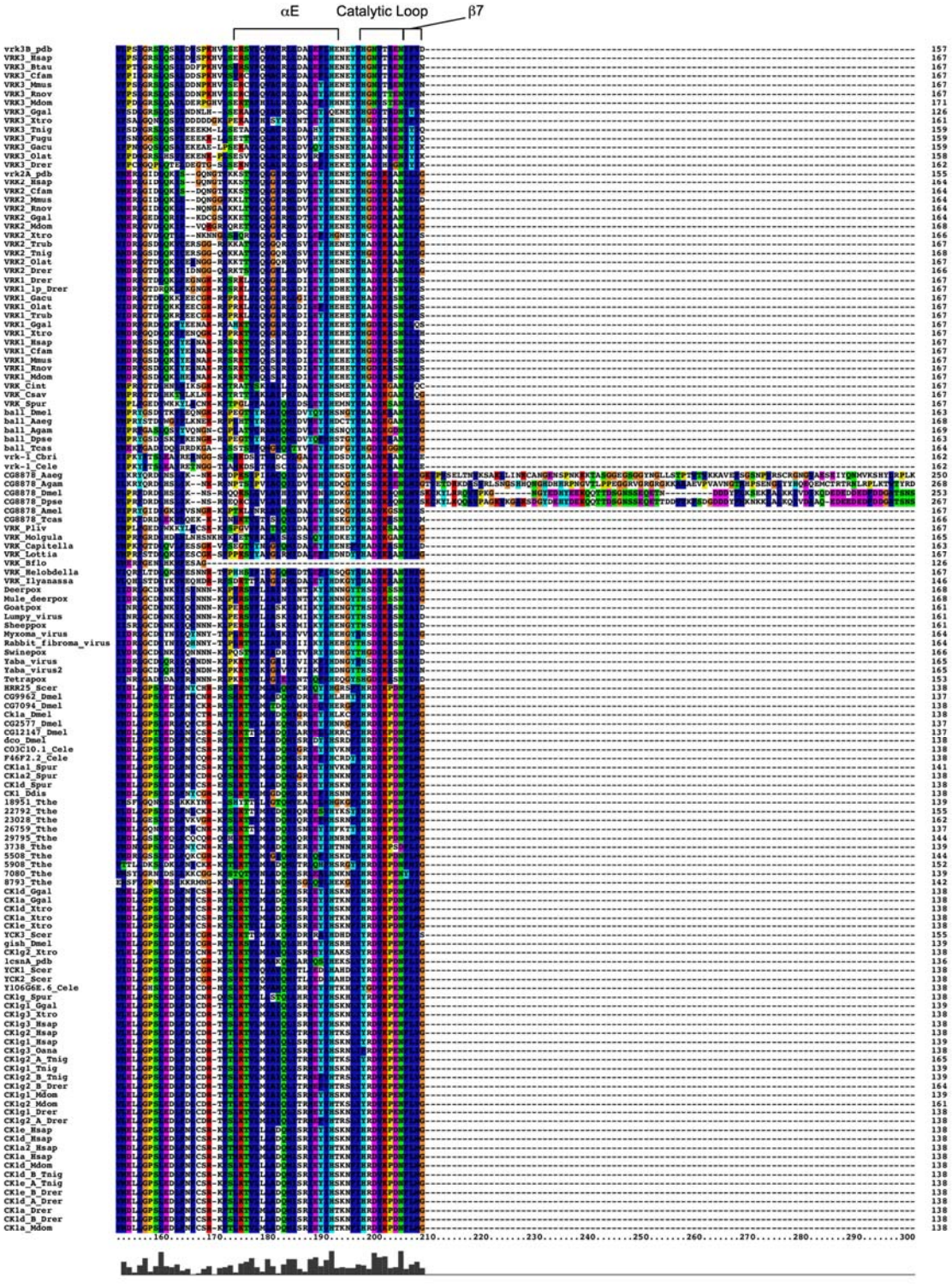
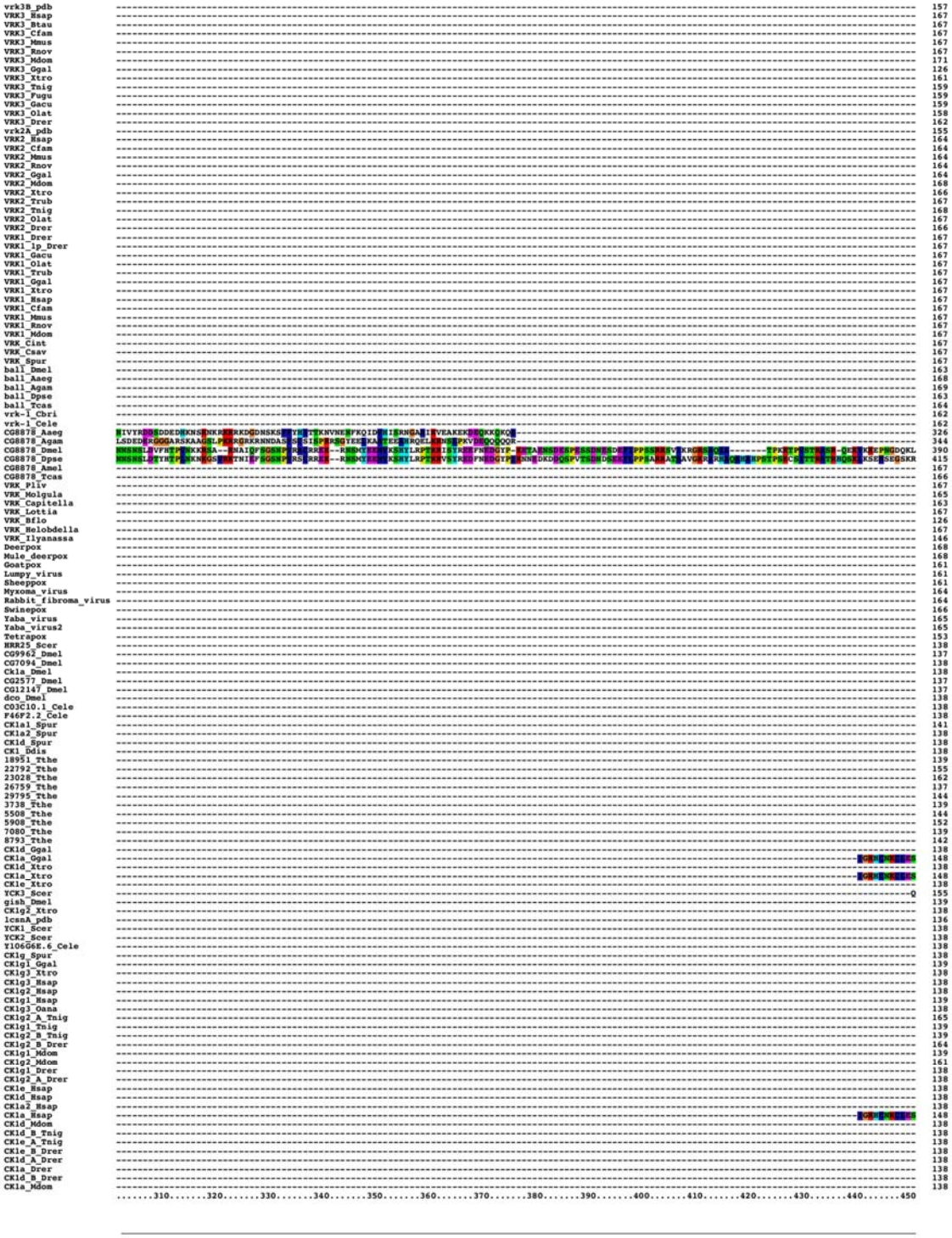
Compound name	Chemical structure	VRK2 Tm Shift [°C]
ASC67		4.5
3-(2-Chloro-3-indolylmethylene)-1,3-dihydroindol-2-one		4
ASC24		4
"Oxindole I; 3-(1H-Pyrrol-2-ylmethylene)-1,3-dihydroindol-2-one"		3.5
ASC29		3.5

Figure S1. Curated Alignment of VRKs and CK1s
Key sections discussed in text are highlighted.




αE
Catalytic Loop
β7


vrk3B_pdb
VRK3_Hsap
VRK3_Btau
VRK3_Cfam
VRK3_Mmus
VRK3_Rnov
VRK3_Mdom
VRK3_Ggal
VRK3_Xtro
VRK3_Tnig
VRK3_Fugu
VRK3_Gacu
VRK3_Olat
VRK3_Drer
vrk2A_pdb
VRK2_Hsap
VRK2_Cfam
VRK2_Mmus
VRK2_Rnov
VRK2_Ggal
VRK2_Mdom
VRK2_Xtro
VRK2_Trub
VRK2_Tnig
VRK2_Olat
VRK2_Drer
VRK1_Drer
VRK1_lp_Drer
VRK1_Gacu
VRK1_Olat
VRK1_Trub
VRK1_Ggal
VRK1_Xtro
VRK1_Hsap
VRK1_Cfam
VRK1_Mmus
VRK1_Rnov
VRK1_Mdom
VRK_Cint
VRK_Csav
VRK_Spur
ball_Dmel
ball_Aaeg
ball_Agam
ball_Dpse
ball_Tcas
vrk-1_Cbri
vrk-1_Cele
CG8878_Aaeg
CG8878_Agam
CG8878_Dmel
CG8878_Dpse
CG8878_Amel
CG8878_Tcas
VRK_Pliv
VRK_Molgula
VRK_Capitella
VRK_Lottia
VRK_Bflo
VRK_Helobdella
VRK_Ilyanassa
Deerpox
Mule_deerpox
Goatpox
Lumpy_virus
Sheeppox
Myxoma_virus
Rabbit_fibroma_virus
Swinepox
Yaba_virus
Yaba_virus2
Tetrapox
HRR25_Scer
CG9962_Dmel
CG7094_Dmel
Cka_Dmel
CG2577_Dmel
CG12147_Dmel
dco_Dmel
C03C10.1_Cele
F46F2.2_Cele
CKla1_Spur
CKla2_Spur
CKld_Spur
CKl_Ddis
18951_Tthe
22792_Tthe
23028_Tthe
26759_Tthe
29795_Tthe
3738_Tthe
5508_Tthe
5908_Tthe
7080_Tthe
8793_Tthe
CKld_Ggal
CKla_Ggal
CKld_Xtro
CKla_Xtro
CKle_Xtro
YCK3_Scer
gish_Dmel
CKlg2_Xtro
1csnA_pdb
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YCK2_Scer
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CKlg1_Ggal
CKlg3_Xtro
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CKlg2_Hsap
CKlg1_Hsap
CKlg3_Oana
CKlg2_A_Tnig
CKlg1_Tnig
CKlg2_B_Tnig
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CKlg1_Mdom
CKlg2_Mdom
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CKlg2_A_Drer
CKle_Hsap
CKld_Hsap
CKla2_Hsap
CKla_Hsap
CKld_Mdom
CKld_B_Tnig
CKle_A_Tnig
CKle_B_Drer
CKld_A_Drer
CKla_Drer
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310
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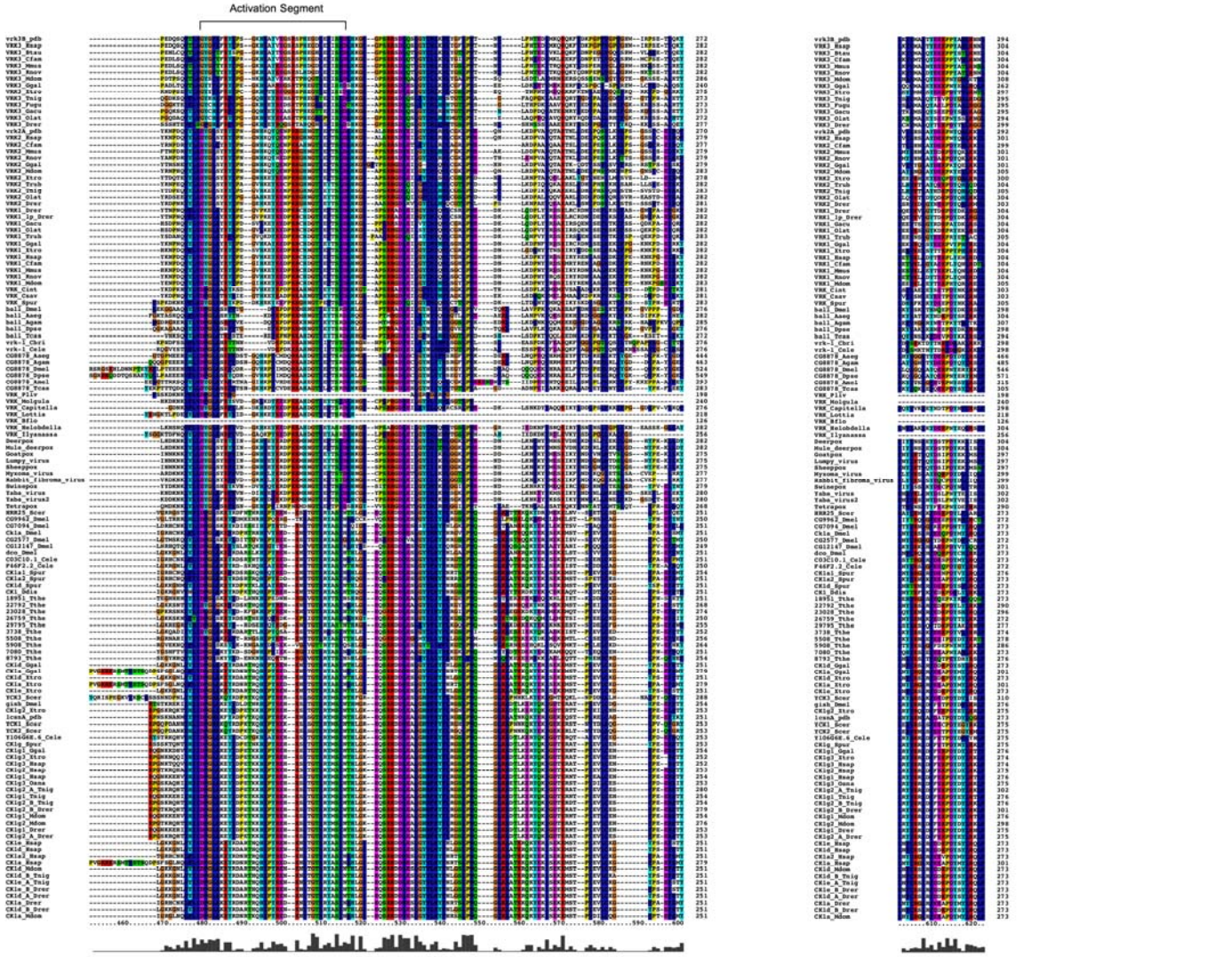
Activation Segment