

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 ₁ 2 ₁ 2	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)
<i>R</i> _{merge} *	0.0562 (0.5859)	0.0648 (0.4167)
<i>I</i> / σI *	15.3 (2.3)	12.5 (2.2)
Refinement		
Reflections (<i>R</i> _{free} set)	64302 (3413)	57853 (3092)
<i>R</i> _{work} / <i>R</i> _{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]	0.015	0.011
RMSD [°]	1.483	1.265
Ramachandran		
favorable [%]	99.6	100.0
allowed [%]	0.4	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 paralogues are highlighted in gray.

Table S3. Inhibitor Screening Results for VRK1

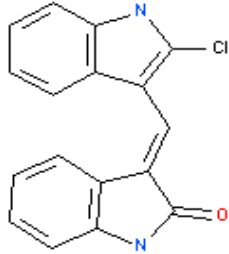
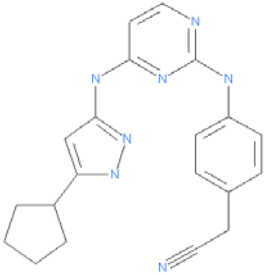
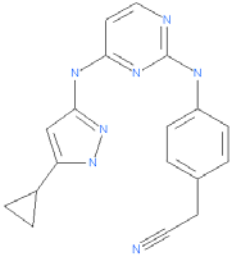
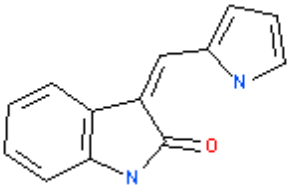
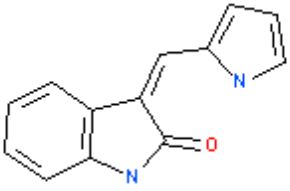
Compound name	Chemical structure	VRK1 T _m Shift [°C]
3-(2-Chloro-3-indolylmethylene)-1,3-dihydroindol-2-one	 <p>The structure shows two indole rings. The top indole ring has a chlorine atom at the 2-position and is connected via its 3-position to the 3-position of a second indole ring. This second indole ring has a carbonyl group at the 2-position.</p>	4
ASC67	 <p>The structure features a central pyrimidine ring connected via its 2 and 6 positions to two imidazole rings. One imidazole ring is substituted with a cyclopentane ring, and the other is substituted with a 4-cyanophenyl group.</p>	4.3
ASC24	 <p>The structure is similar to ASC67, with a central pyrimidine ring connected to two imidazole rings. One imidazole ring is substituted with a cyclopropane ring, and the other is substituted with a 4-cyanophenyl group.</p>	4.5
Oxindole I; 3-(1H-Pyrrol-2-ylmethylene)-1,3-dihydroindol-2-one	 <p>The structure shows an indole ring with a carbonyl group at the 2-position, which is connected via its 3-position to the 2-position of a pyrrole ring.</p>	3.5
5-Iodo-3-[(3,5-dibromo-4-hydroxyphenyl)methylene]-2-indolinone	 <p>The structure shows an indole ring with a carbonyl group at the 2-position, which is connected via its 3-position to the 4-position of a phenyl ring. The phenyl ring has bromine atoms at the 3 and 5 positions and a hydroxyl group at the 4 position.</p>	3.5

Table S4. Inhibitor Screening Results for VRK2

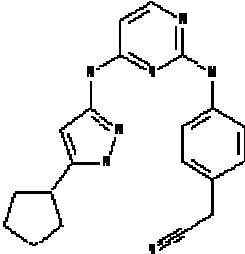
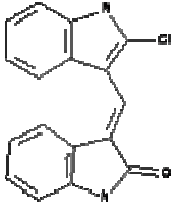
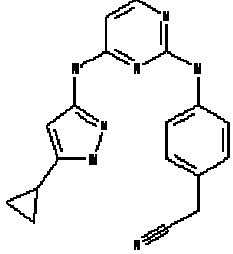
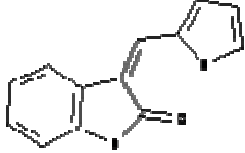
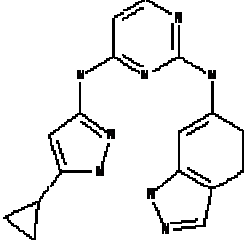
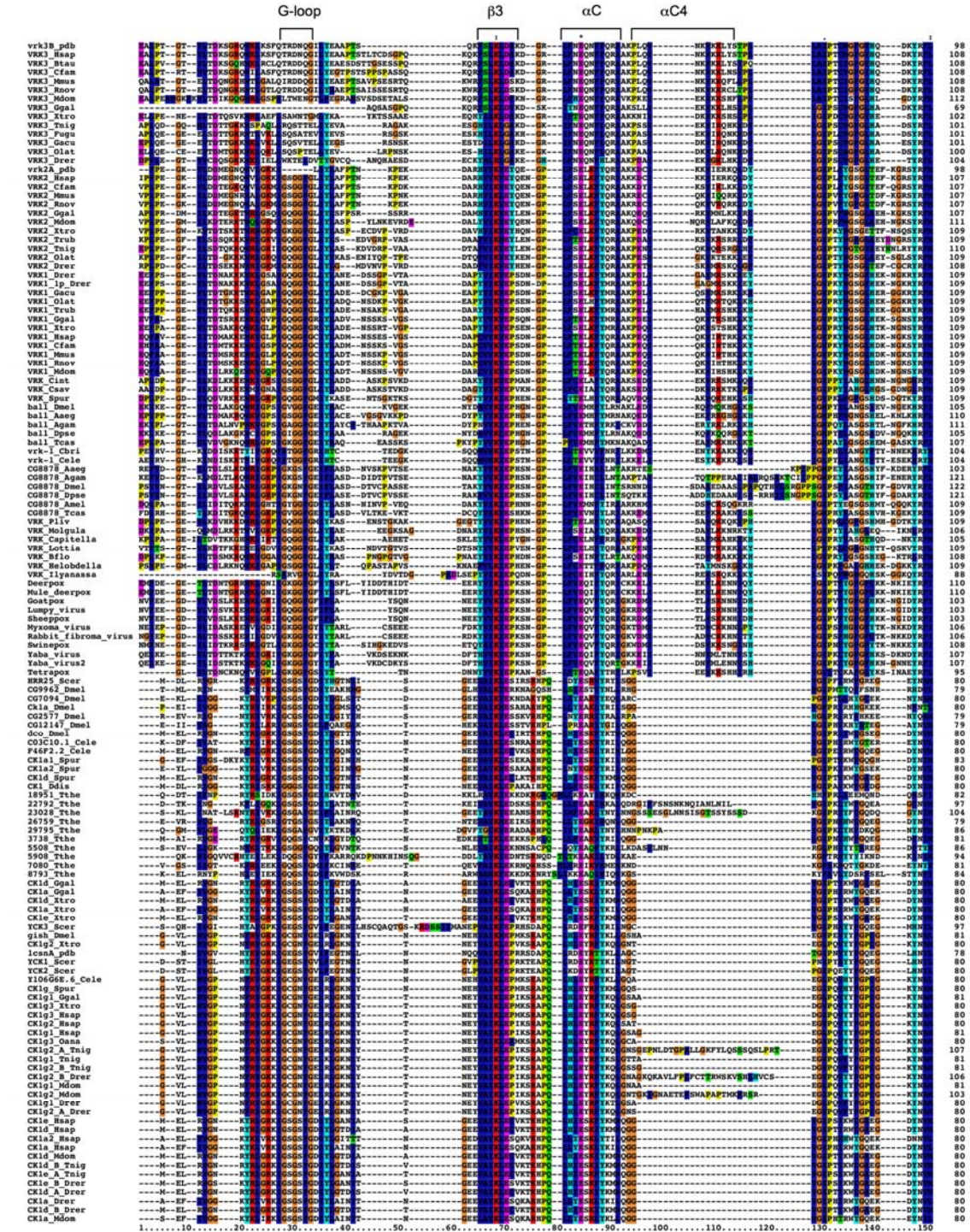
Compound name	Chemical structure	VRK2 T _m Shift [°C]
ASC67		4.5
3-(2-Chloro-3-indolylmethylene)- 1,3-dihydroindol-2-one		4
ASC24		4
"Oxindole I; 3-(1H-Pyrrol-2-ylmethylene)-1,3-dihydroindol-2-one"		3.5
ASC29		3.5

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



vrk3B_pdb	157
VRK3_Hsap	167
VRK3_Btau	167
VRK3_Cfam	167
VRK3_Mmus	167
VRK3_Rnov	167
VRK3_Hdom	171
VRK3_Ggal	126
VRK3_Xtro	161
VRK3_Tnig	159
VRK3_Pugu	159
VRK3_Gacu	159
VRK3_Olat	158
VRK1_Drer	162
vrk2A_pdb	155
VRK2_Hsap	164
VRK2_Cfam	164
VRK2_Mmus	164
VRK2_Rnov	164
VRK2_Ggal	164
VRK2_Hdom	168
VRK2_Xtro	166
VRK2_Trub	167
VRK2_Tnig	168
VRK2_Olat	167
VRK2_Drer	166
VRK1_Drer	167
VRK1_Ip_Drer	167
VRK1_Gacu	167
VRK1_Olat	167
VRK1_Trub	167
VRK1_Ggal	167
VRK1_Xtro	167
VRK1_Hsap	167
VRK1_Cfam	167
VRK1_Mmus	167
VRK1_Rnov	167
VRK1_Hdom	167
VRK_Cint	167
VRK_Csav	167
VRK_Spur	167
ball_Dmel	163
ball_Aaeg	168
ball_Agam	169
ball_Dpse	163
ball_Tcas	164
vrk-1_Cbri	162
vrk-1_Cele	162
CG878_Aaeg	326
CG878_Agam	344
CG878_Dmel	360
CG878_Dpse	415
CG878_Amel	167
CG878_Tcas	166
VRK_Pliv	167
VRK_Molpula	165
VRK_Capitella	163
VRK_Lottia	167
VRK_Bilo	126
VRK_Melobdella	167
VRK_Ilyanassa	146
Deerpox	168
Mula_deerpox	168
Goatpox	161
Lumpy_virus	161
Ebspox	161
Myxoma_virus	164
Rabbit_fibroma_virus	164
Swinepox	166
Yaba_virus	165
Yaba_virus2	165
Tatrapox	165
RRR25_Scer	138
CG9962_Dmel	137
CG7094_Dmel	138
Ck1a_Dmel	138
CG2577_Dmel	137
CG12147_Dmel	137
cco_Dmel	138
CG10101_Cele	138
F46F2.2_Cele	138
CK1a1_Spur	141
CK1a2_Spur	138
CK1d_Spur	138
CK1_Ddis	138
18951_Ttbe	139
22792_Ttbe	155
23028_Ttbe	162
26759_Ttbe	137
29795_Ttbe	144
3738_Ttbe	139
5508_Ttbe	144
5908_Ttbe	152
7080_Ttbe	139
8753_Ttbe	142
CK1d_Ggal	138
CK1a_Ggal	138
CK1d_Xtro	138
CK1a_Xtro	148
CK1a_Xtro	148
YCK3_Scer	155
gish_Dmel	139
CK1g_Xtro	138
IcanA_pdb	136
YCK1_Scer	138
YCK2_Scer	138
Y106668.6_Cele	138
CK1g_Spur	138
CK1g1_Ggal	139
CK1g1_Xtro	138
CK1g1_Hsap	138
CK1g2_Hsap	138
CK1g1_Hsap	139
CK1g3_Oma	138
CK1g2_A_Tnig	165
CK1g1_Tnig	139
CK1g2_B_Tnig	139
CK1g2_B_Drer	144
CK1g1_Hdom	139
CK1g2_Hdom	161
CK1g1_Drer	138
CK1g2_A_Drer	138
CK1e_Hsap	138
CK1d_Hsap	138
CK1a2_Hsap	138
CK1a_Hsap	148
CK1d_Mdom	138
CK1d_B_Tnig	138
CK1a_A_Tnig	138
CK1e_B_Drer	138
CK1d_A_Drer	138
CK1a_Drer	138
CK1d_B_Drer	138
CK1a_Hdom	138

.....310.....320.....330.....340.....350.....360.....370.....380.....390.....400.....410.....420.....430.....440.....450

Activation Segment

