

SUPPLEMENTARY INFORMATION

Table S1. Likelihood ratio test statistics for models of variable selective pressure along phylogenetic branches of primate PKR.

	ℓ	2δ	df*	P value
Model 0 same dN/dS ratio for all branches	-5729.03			
Model 1 different dN/dS ratio for each branch	-5686.93	84.21	39	<0.0003

* denotes degrees of freedom that is equal to one less than the total number of branches in the phylogeny.

Table S2. Summary of positive selection in Protein Kinase R

PKR	codon freq.	<i>M1-M2</i>		<i>M7-M8</i>		<i>M8a-M8</i>		tree length	dN/dS (%)
		2 δ	p- value	2 δ	p- value	2 δ	p- value		
whole protein (1-551)	f61	74.7	p<0.0001	75.6	p<0.0001	74.3	p<0.0001	1.21	4.1 (15%)
	f3x4	71.4	p<0.0001	71.9	p<0.0001	71.0	p<0.0001	1.20	3.7 (17%)
kinase domain (265-551)	f61	71.7	p<0.0001	71.7	p<0.0001	71.5	p<0.0001	1.16	7.0 (11%)
	f3x4	58.1	p<0.0001	58.2	p<0.0001	58.0	p<0.0001	1.12	5.5 (11%)
spacer region (167-264)	f61	17.1	p<0.0002	25.1	p<0.0001	17.1	p<0.0001	1.70	3.6 (30%)
	f3x4	22.3	p<0.0001	18.8	p<0.0001	18.3	p<0.0001	1.64	3.5 (38%)
dsRNA binding domains (55-166)	f61	4.8	p<0.0894	4.9	p<0.0848	4.8	p<0.0280	1.11	2.1 (36%)
	f3x4	2.0	p<0.3639	2.3	p<0.3139	2.0	p<0.1552	1.07	1.5 (41%)

Table S3. PKR whole gene log likelihood scores and parameter estimates for five models of variable ω among sites assuming the f61 model of codon frequencies.

Site model	Parameter estimates	Sites* with $\omega^{**}>1$	ℓ
M1: neutral	$(\omega_0=0) f_0=0.461$ $(\omega_1=1) f_1=0.539$ branch ω (mean)=0.553		-5664.28
M2: selection	$(\omega_0=0.1) f_0=0.452$ $(\omega_1=1) f_1=0.397$ $(\omega_2=4.0) f_2=0.151$ branch ω (mean)=1.042	7 A 0.929 122 A 0.962 123 S 0.918 206 G 0.969 224 S 0.981 242 S 0.978 259 V 0.996 261 K 0.982 265 M 0.992 344 S 1.000 351 S 0.955 376 Q 0.907 380 K 0.992 462 S 0.902 489 F 0.998 496 T 0.999 502 I 0.976 503 I 0.923 538 V 0.949	-5626.94
M7: β	$p=0.00759$ $q=0.00500$ branch ω (mean)=0.600		-5664.95
M8a: β and $\omega=1$	$p=3.16777$ $q=99.00000$ ($f_0=0.460$) $\omega_1=1$ ($f_1=0.540$) branch ω (mean)=0.554		-5664.30
M8: β and ω	$p=0.12863$ $q=0.11990$ ($f_0=0.849$) $\omega_1=4.1$ ($f_1=0.151$) branch ω (mean)=1.050	6 S 0.901 7 A 0.967 24 V 0.936 44 Q 0.917 49 G 0.942 86 L 0.929 122 A 0.985 123 S 0.957 125 V 0.933 139 Q 0.944 185 T 0.935 206 G 0.988 242 S 0.991 255 T 0.982 259 V 0.999 261 K 0.993 265 M 0.997 275 S 0.944 322 H 0.935 330 F 0.947 336 T 0.939 338 D 0.942	-5627.16

		344 S	1.000	
		351 S	0.980	
		376 Q	0.957	
		380 K	0.997	
		462 S	0.947	
		489 F	0.999	
		492 S	0.920	
		496 T	1.000	
		502 I	0.989	
		506 I	0.964	
		516 Q	0.933	
		538 V	0.977	

* sites with posterior probabilities >0.90 by Bayes Empirical Bayes (BEB) analysis

** $\omega = (dN/dS)$

Table S4. Likelihood ratio test statistics for PARRIS analysis of PKR whole gene.

	ℓ	2δ	P value	Parameter estimates
Null model (M1): no selection	-5637.1			
Alternative model (M2): selection	-5618.0	38	<0.0001	$\omega_0=0.14$ ($f_0=0.449$) $\omega_1=1.00$ ($f_1=0.173$) $\omega_2=2.31$ ($f_2=0.378$)

Table S5. Likelihood ratio test statistics for PARRIS analysis of PKR whole gene taking into account one recombination breakpoint supported by GARD analysis*.

	ℓ	2δ	P value	Parameter estimates
Null model (M1): no selection	-7147.1			
Alternative model (M2): selection	-7135.1	24	<0.0001	$\omega_0=0.48$ ($f_0=0.430$) $\omega_1=1.00$ ($f_1=0.307$) $\omega_2=2.22$ ($f_2=0.263$)

*GARD predicted a breakpoint at nucleotide 1006 (Δ c-AIC = 27.02).

Table S6. Summary of lineage specific positive selection in Protein Kinase R (PAML)

PKR	codon freq.	<i>M1-M2</i>		<i>M7-M8</i>		<i>M8a-M8</i>		tree length	dN/dS (%)
		2 δ	p- value	2 δ	p- value	2 δ	p- value		
hominoid 8 species	f61	12.5	p<0.002	12.5	p<0.002	12.5	p<0.0005	0.24	3.1 (32%)
	f3x4	11.9	p<0.003	13.1	p<0.002	11.9	p<0.0006	0.24	3.0 (33%)
old world monkey 7 species	f61	77.3	p<0.0001	77.6	p<0.0001	77.3	p<0.0001	0.38	9.2 (15%)
	f3x4	82.0	p<0.0001	82.4	p<0.0001	82.0	p<0.0001	0.39	9.7 (15%)
new world monkey 6 species	f61	18.1	p<0.0002	18.3	p<0.0002	18.1	p<0.0001	0.39	5.4 (8%)
	f3x4	17.4	p<0.0002	17.8	p<0.0002	17.4	p<0.0001	0.39	5.4 (7%)

Table S7. PKR spacer region log likelihood scores and parameter estimates for five models of variable ω among sites assuming the f61 model of codon frequencies.

Site model	Parameter estimates	Sites with $\omega > 1$	ℓ
M1: neutral	$(\omega_0=0) f_0=0.201$ $(\omega_1=1) f_1=0.799$ branch ω (mean)=0.799		-1192.00
M2: selection	$(\omega_0=0.0) f_0=0.147$ $(\omega_1=1) f_1=0.543$ $(\omega_2=3.5) f_2=0.310$ branch ω (mean)=1.633	206 G 0.977 224 S 0.920 242 S 0.981 255 T 0.957 259 V 0.997 261 K 0.984	-1183.43
M7: β	$p=15.07816$ $q=0.00500$ branch ω (mean)=1.000		-1195.98
M8a: β and $\omega=1$	$p=0.00500$ $q=1.60970 (f_0=0.201)$ $\omega_1=1 (f_1=0.799)$ branch ω (mean)=0.799		-1192.00
M8: β and ω	$p=0.01990$ $q=0.00500 (f_0=0.697)$ $\omega_1=3.6 (f_1=0.303)$ branch ω (mean)=1.638	185 T 0.935 206 G 0.991 224 S 0.967 242 S 0.992 255 T 0.983 259 V 0.998 261 K 0.993	-1183.43

Table S8. PKR kinase domain log likelihood scores and parameter estimates for five models of variable ω among sites assuming the f61 model of codon frequencies.

Site model	Parameter estimates	Sites with $\omega > 1$	ℓ
M1: neutral	$(\omega_0=0.01) f_0=0.483$ $(\omega_1=1) f_1=0.517$ branch ω (mean)=0.523		-2789.37
M2: selection	$(\omega_0=0) f_0=0.386$ $(\omega_1=1) f_1=0.507$ $(\omega_2=6.7) f_2=0.107$ branch ω (mean)=1.228	265 M 0.998 275 S 0.932 322 H 0.923 330 F 0.935 336 T 0.936 338 D 0.922 344 S 1.000 351 S 0.980 376 Q 0.912 380 K 0.998 462 S 0.932 489 F 1.000 492 S 0.905 496 T 1.000 502 I 0.993 506 I 0.946 516 Q 0.900 538 V 0.974	-2753.52
M7: β	$p=0.01911$ $q=0.01698$ branch ω (mean)=0.509		-2789.47
M8a: β and $\omega=1$	$p=0.04150$ $q=3.80019$ ($f_0=0.474$) $\omega_1=1$ ($f_1=0.526$) branch ω (mean)=0.529		-2789.40
M8: β and ω	$p=0.00762$ $q=0.00507$ ($f_0=0.894$) $\omega_1=7.0$ ($f_1=0.106$) branch ω (mean)=1.274	265 M 0.999 275 S 0.965 322 H 0.961 330 F 0.974 336 T 0.967 338 D 0.970 344 S 1.000 351 S 0.991 376 Q 0.966 380 K 0.999 462 S 0.964 489 F 1.000 492 S 0.951 496 T 1.000 502 I 0.997 506 I 0.978 516 Q 0.949 538 V 0.989	-2753.63

Table S9. Spidermonkey/BGM analysis of co-evolution between amino acid positions in PKR phylogeny ($P^* > 0.5$).**

Site 1***	Site 2***	P*	notes
E379	S492	0.65	492 positive selection, 379 contacts eIF2 α
S6	Q376	0.66	both under positive selection, 376 in helix α D
K261	T336	0.59	both under positive selection
A7	S192	0.70	7 under positive selection

* posterior probability for site 1 and site 2 being conditionally dependent

** one parent undirected analysis for all variable sites with a threshold of 3 branches with non-synonymous substitutions

*** amino acid shown for each position is from human PKR

Table S10. Primers used to amplify primate PKR.

Primer	Sequence
PKR-F1	5'-CTGTGATTATCTGCGTGCATTTTGG-3'
PKR-R1	5'-CTAAGGAAAATTGCATATCAGAAGCAGG-3'
PKR-F2	5'-GAAATGGCTGGTGATCTTTCAGCAGG-3'
PKR-R2	5'-GGATACTTTTCAGAAGGGCTCTAACA-3'

Table S11. Genotypes of yeast strains used in this study.

Strain	Genotype	Source
H2557	<i>MATα ura3-51 leu2-3 leu2-112 gcn2Δ</i>	Ref. 21
J223	<i>MATα ura3-51 leu2-3 leu2-112 trp1-Δ63 gcn2Δ SUI2-S51A</i>	Ref. 21
HM1	<i>MATα ura3-51 leu2-3 leu2-112 gcn2Δ (GAL-CYC1-K3L LEU2) at leu2</i>	this study
HM2	<i>MATα ura3-51 leu2-3 leu2-112 gcn2Δ (GAL-CYC1-HA-K3L LEU2) at leu2</i>	this study
HM3	<i>MATα ura3-51 leu2-3 leu2-112 gcn2Δ (GAL-CYC1 LEU2) at leu2</i>	this study
HM4	<i>MATα ura3-51 leu2-3 leu2-112 gcn2Δ (GAL-CYC1-K3L-variola LEU2) at leu2</i>	this study