Supplementary Material for

Rapid evolution of protein kinase PKR alters sensitivity to viral inhibitors

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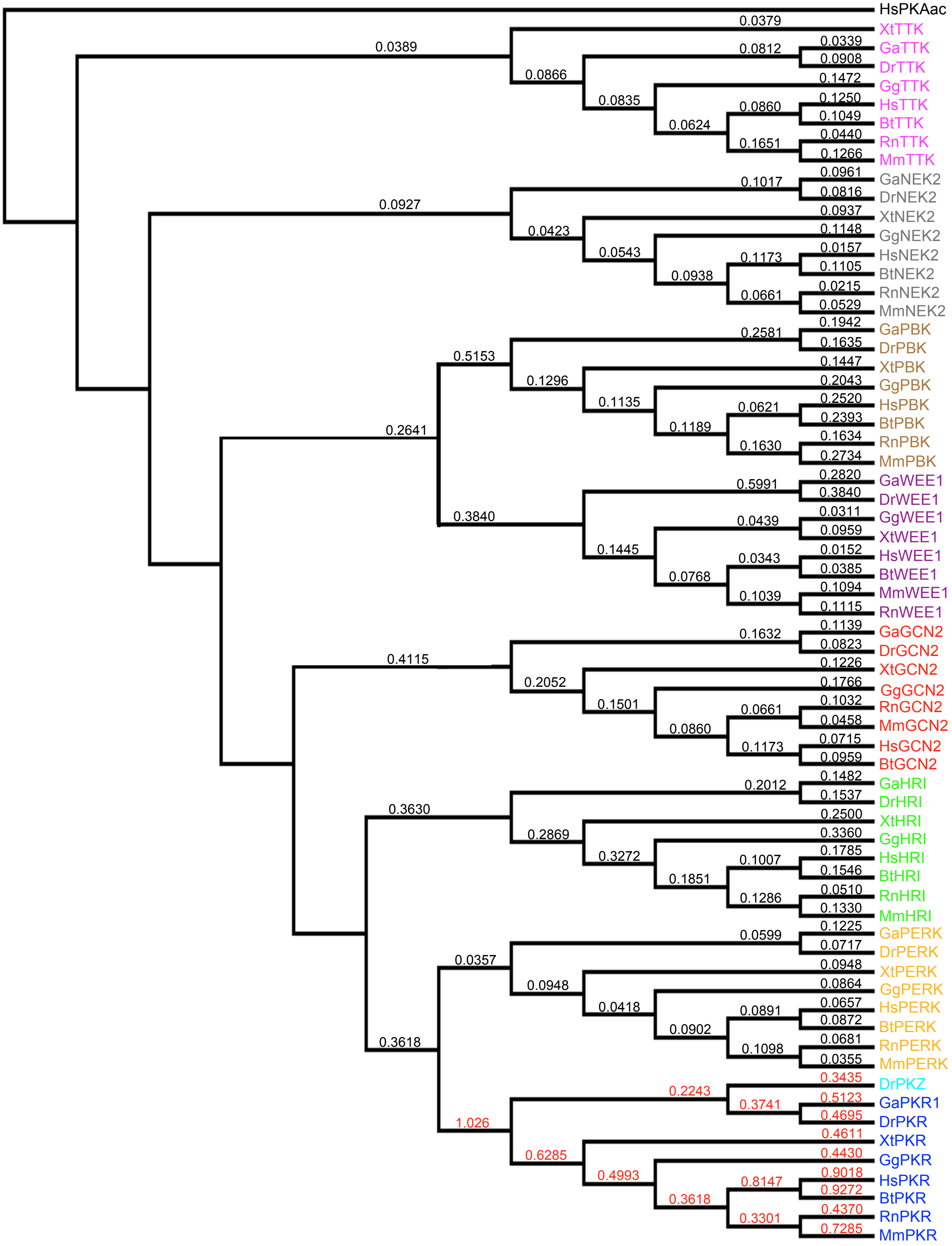
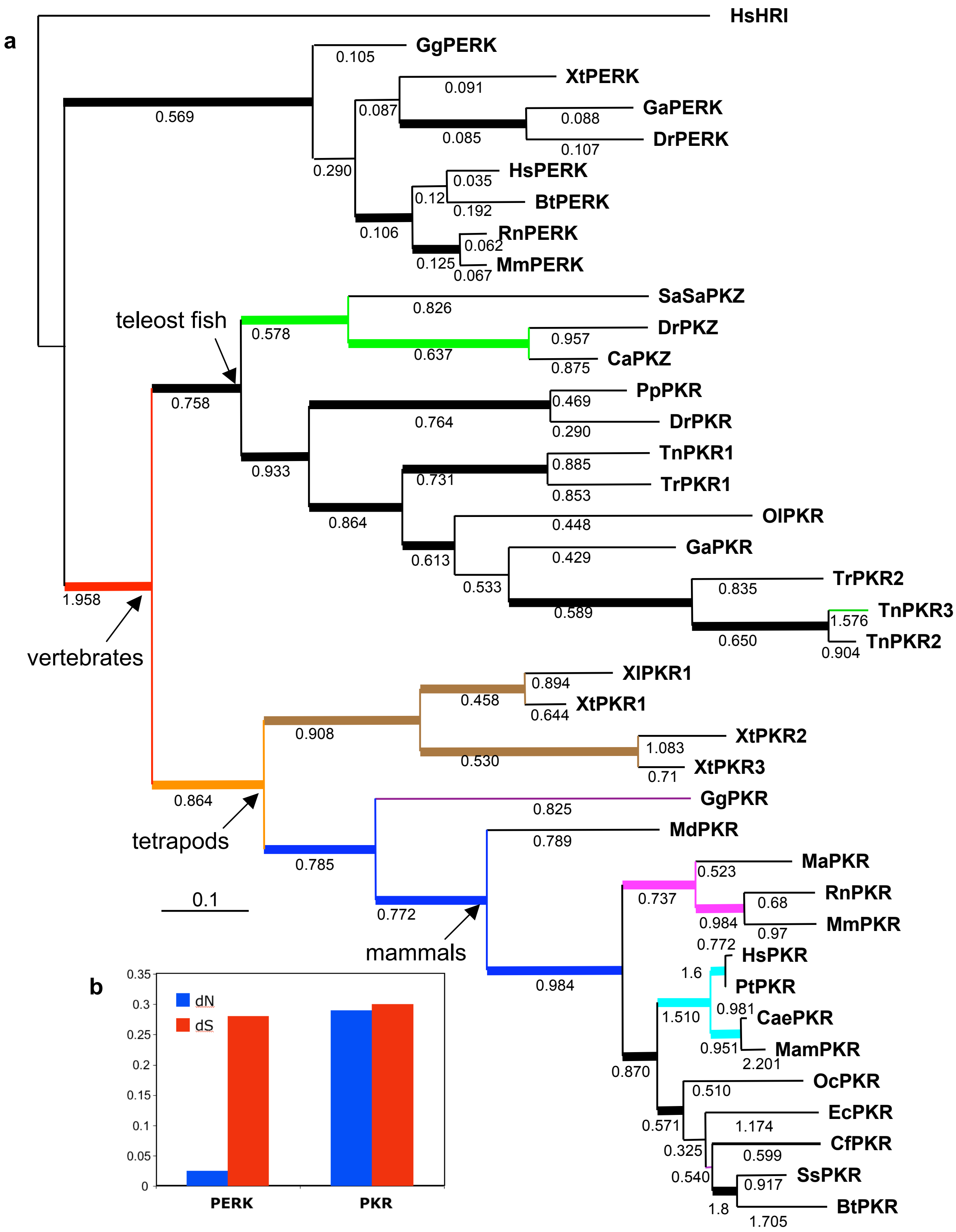


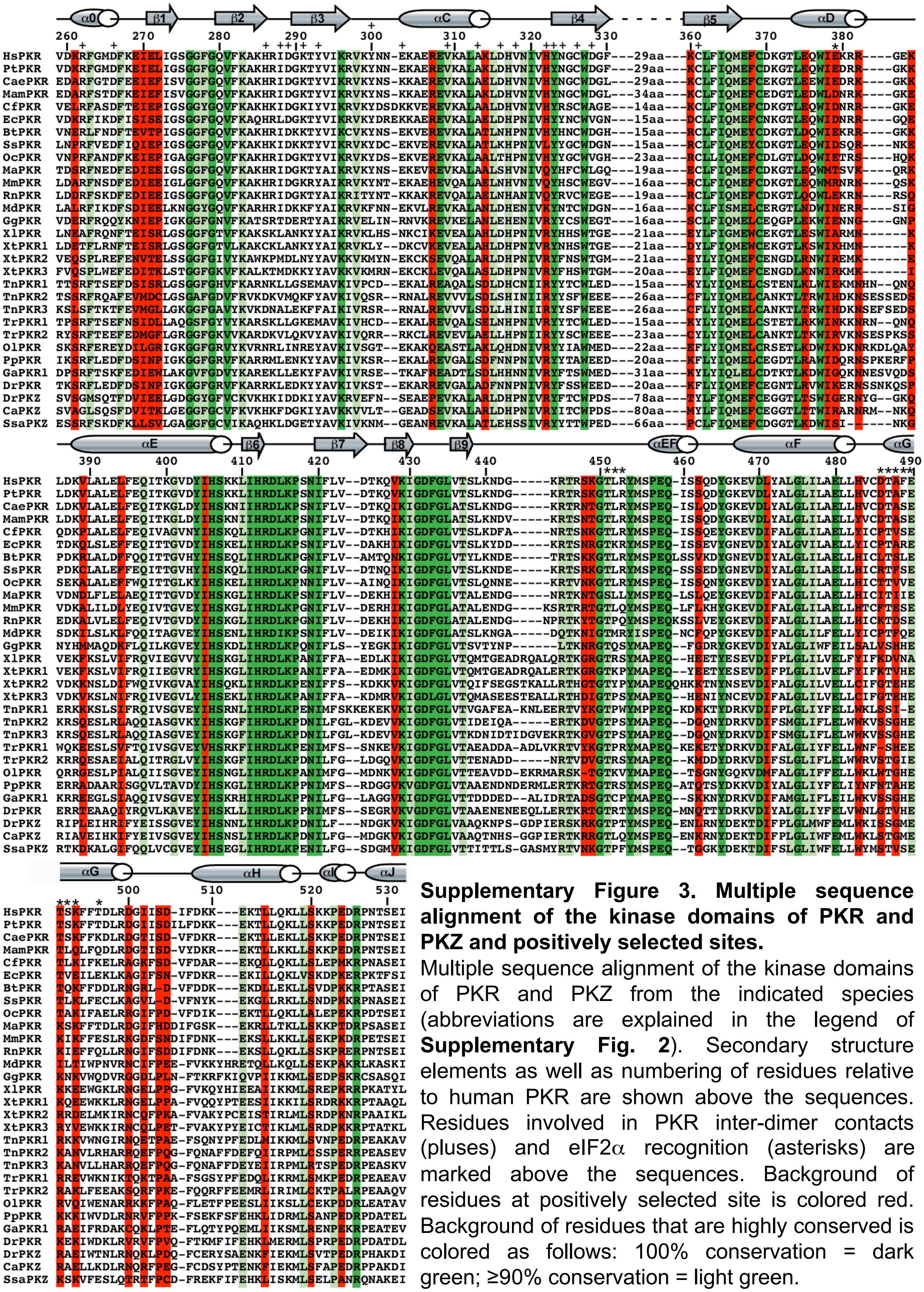
Supplementary Figure 1. Maximum Likelihood topology of the eIF2α- and other kinase domains.

Maximum Likelihood topology of the eIF2α- and other kinase domains (color-coded as in Fig. 1) from human (*Homo sapiens*, Hs), cattle (*Bos taurus*, Bt), mouse (*Mus musculus*, Mm), rat (*Rattus norvegicus*, Rn), chicken (*Gallus gallus*, Gg), frog (*Xenopus tropicalis*, Xt), stickleback fish (*Gasterosteus aculeatus*, Ga) and zebrafish (*Danio rerio*, Dr). HsPKAac is the outgroup. Numbers above branches denote ratios of non-synonymous to synonymous (*dN/dS*) changes.



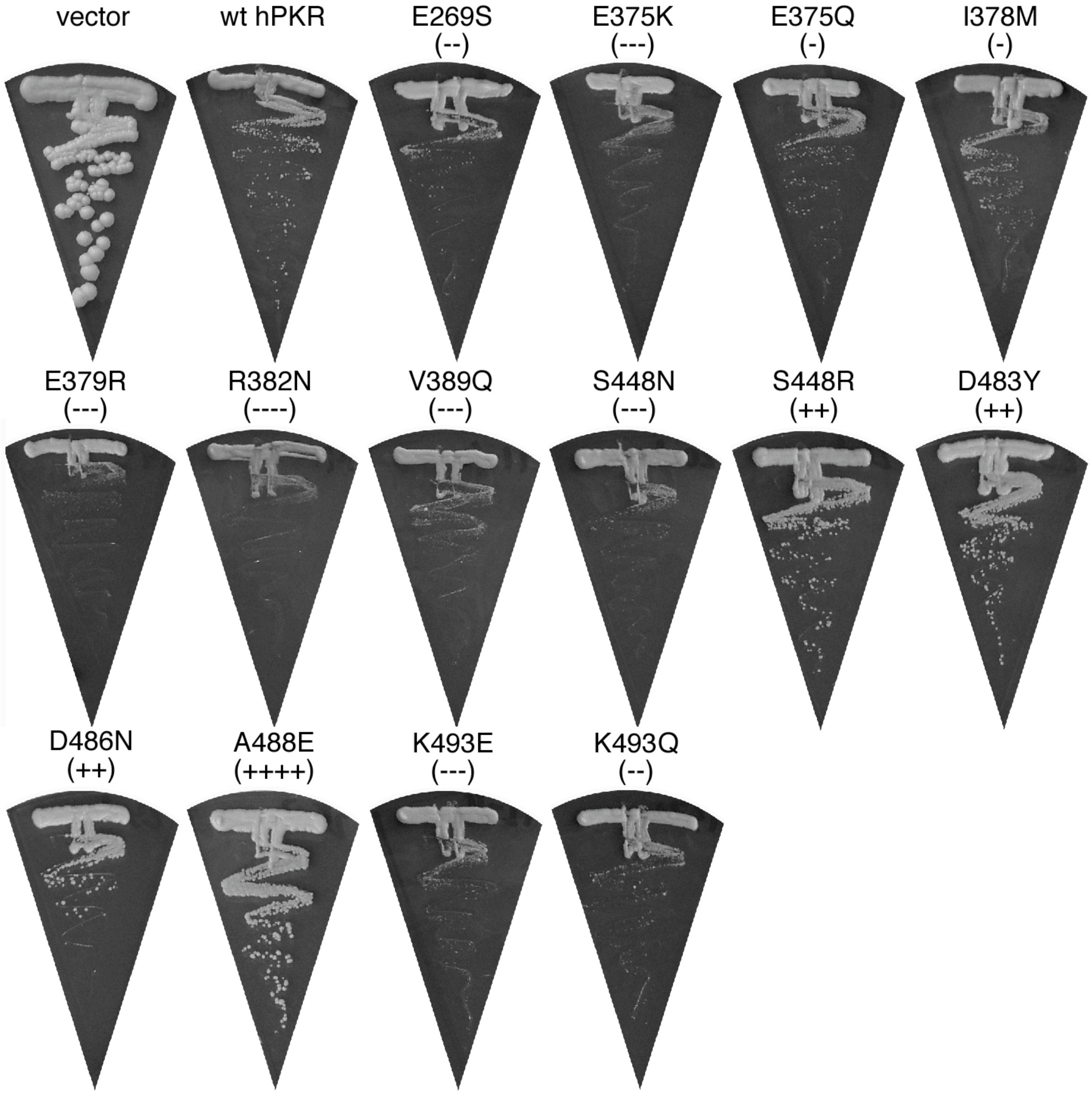
Supplementary Figure 2. Maximum Likelihood topology of the PKR/PKZ family.

(**a**) HsHRI was used as the outgroup. Numbers below the branches denote *dN/dS* ratios. Branches in w positively selected residues have been detected are colored. Color-coding corresponds to residues in Figure 1c, Supplementary Table 2. Thick branches show bootstrap support values of > 75; [-lnL=20189.1]. The follo abbreviations were used: Bt, *Bos taurus* (cattle); Ss, *Sus scrofa* (pig); Cf, *Canis familiaris* (dog); Oc, *Oryctol* *cuniculus* (European rabbit); Ec, *Equus caballus* (horse); Mam, *Macaca mulatta* (Rhesus macaque); (*Chlorocebus aethiops* (African green monkey); Pt, *Pan troglodytes* (chimpanzee); Hs, *Homo sapiens* (human); *Mus musculus* (house mouse); Rn, *Rattus norvegicus* (Norway rat); Ma, *Mesocricetus auratus* (golden hamster); *Monodelphis domestica* (gray short-tailed opossum); Gg, *Gallus gallus* (chicken); Xt, *Xenopus tropicalis* (wes clawed frog); Xl, *Xenopus laevis* (African clawed frog); Tn, *Tetraodon nigroviridis* (green spotted pufferfish) *Takifugu rubripes* (torafugu); Ga, *Gasterosteus aculeatus* (three spined stickleback); Ol, *Oryzias latipes* (Japa medaka); Dr, *Danio rerio* (zebrafish); Pp, *Pimephales promelas* (fathead minnow); Ca, *Carassius auratus* (gold Ssa, *Salmo salar* (Atlantic salmon) (**b**) Column graph of average values of dS (red), and dN (blue) in the sistergr of PERK and PKR kinases. PKR kinases show a significant increase in dN values.



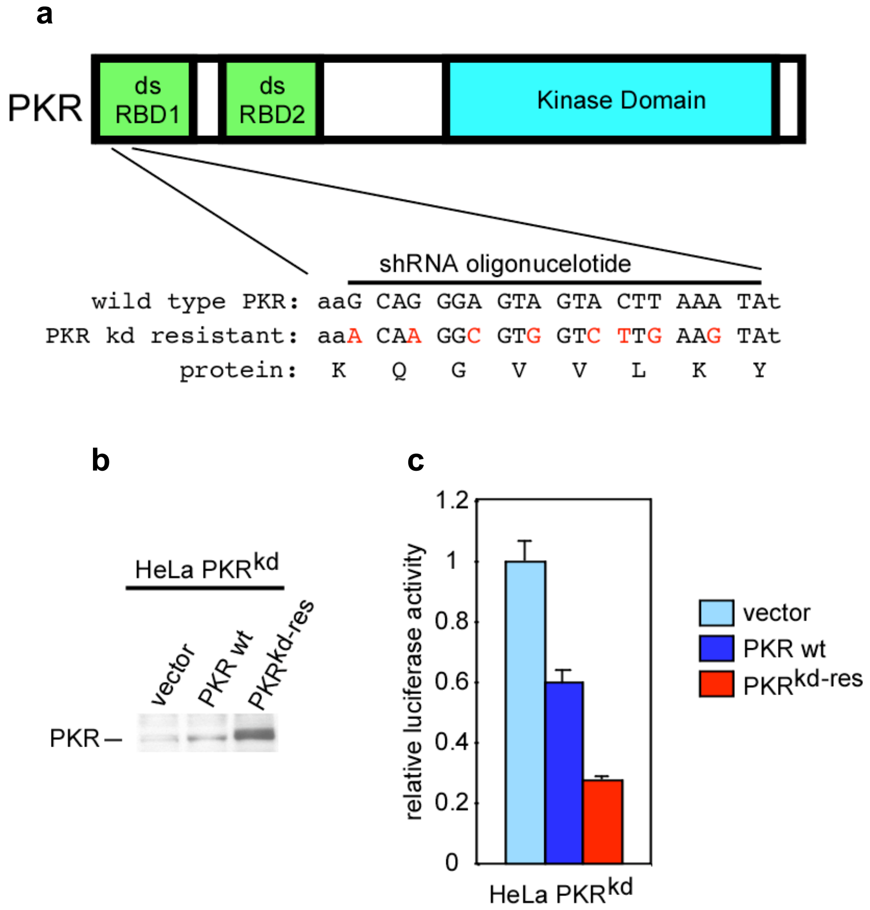
Supplementary Figure 3. Multiple sequence alignment of the kinase domains of PKR and PKZ and positively selected sites.

Multiple sequence alignment of the kinase domains of PKR and PKZ from the indicated species (abbreviations are explained in the legend of **Supplementary Fig. 2**). Secondary structure elements as well as numbering of residues relative to human PKR are shown above the sequences. Residues involved in PKR inter-dimer contacts (pluses) and eIF2α recognition (asterisks) are marked above the sequences. Background of residues at positively selected site is colored red. Background of residues that are highly conserved is colored as follows: 100% conservation = dark green; ≥90% conservation = light green.

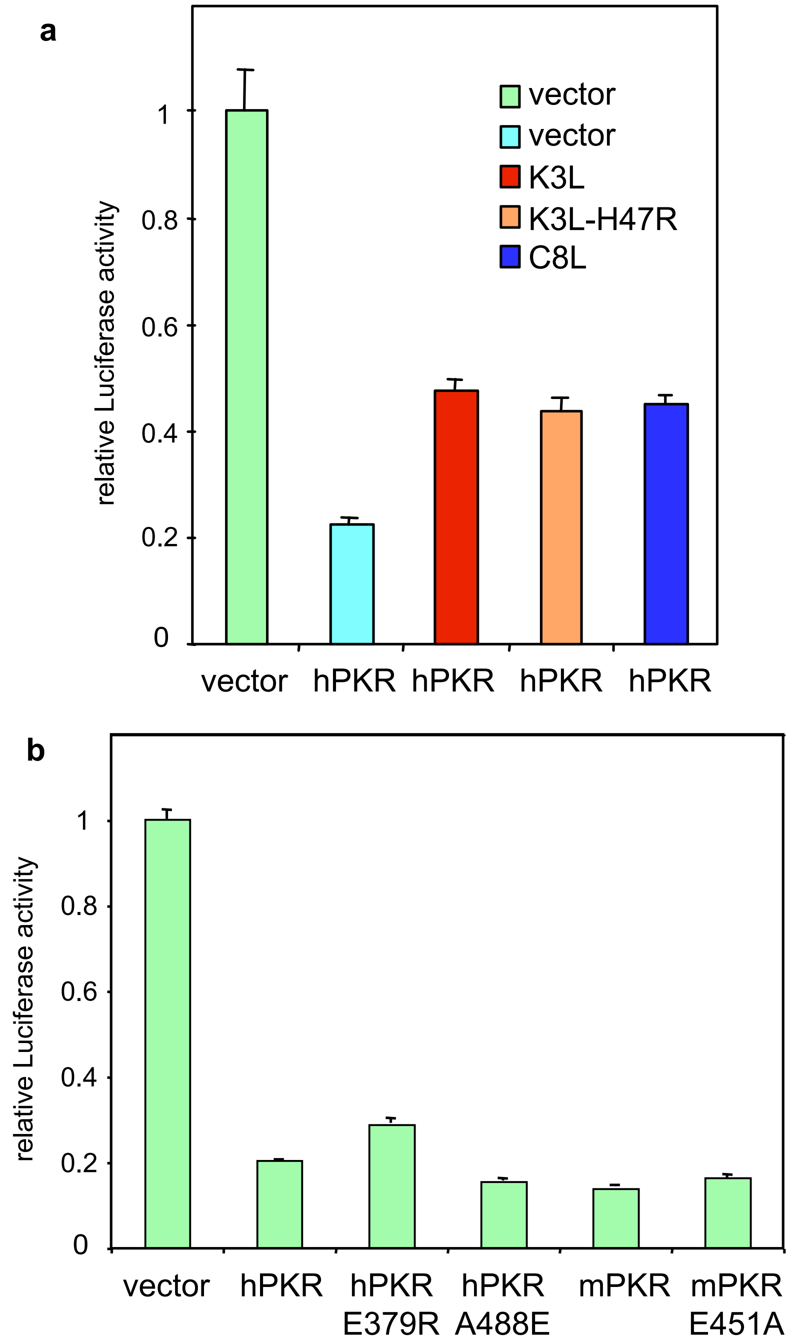


Supplementary Figure 4. Sensitivity of human PKR and PKR mutants to variola virus C3L.

Plasmids expressing WT or the indicated PKR mutants under the control of a galactose-inducible promoter were introduced into a yeast strain expressing varC3L, the homolog of vacK3L. Transformants were streaked on galactose medium and incubated 4 days at 30˚C. Mutants that exhibited altered sensitivity to C3L as compared with WT PKR are shown. Growth of colonies was evaluated and is indicated: (-) to (----) for poor growth of yeast relative to transformants expressing WT PKR indicating weak to strong resistance to C3L, and (+) to (++++) for better growth than yeast expressing WT PKR indicating weakly to strongly increased sensitivity to C3L.



Supplementary Figure 5. Construction of knock-down resistant PKR
(**a**) Schematic representation of the PKR domain organization (dsRBD = double-stranded RNA binding domains). The relative position of the target sequence for the shRNA is indicated. Synonymous sites in the shRNA target sequence were mutated (shown in red) to obtain knock-down (kd) resistant PKR ($\text{PKR}^{\text{kd-res}}$). (**b**) Immunoblot analysis of PKR expression in HeLa PKR^{kd} cells. Cells were transfected with vector, wild type hPKR or $\text{hPKR}^{\text{kd-res}}$. (**c**) HeLa PKR^{kd} cells were cotransfected with a luciferase reporter vector and vector, wild type hPKR or $\text{hPKR}^{\text{kd-res}}$. Relative luciferase activity, normalized to the vector transfection, is shown. Standard deviations are indicated for three independent transfections.



Supplementary Figure 6. Inhibition of luciferase by PKR
(**a**) HeLa PKR^{kd} cells were cotransfected with a luciferase reporter, human hPKR$^{kd\text{-}res}$ and the indicated K3L orthologs: vacK3L, vacK3L-H47R and swinepox C8L. Luciferase activity was normalized to vector (control) transfections. Standard deviations are indicated for three independent transfections. (**b**) HeLa PKR^{kd} cells were cotransfected with a luciferase reporter and vector, knock-down resistant derivatives of hPKR, hPKR-E379R, hPKR-A488E, mPKR or mPKR-E451A.

	PKR	PERK *	HRI*	GCN2*	Wee1*	PBK*	Nek2*	TTK*
Hs	0.28808	0.02985	0.1312	0.03646	0.0945	0.1241	0.06743	0.0779
Bt	0.29472	0.03953	0.1226	0.03645	0.0937	0.1245	0.06771	0.0975
Rn	0.27931	0.03645	0.1289	0.02906	0.101	0.1363	0.064074	0.1166
Mm	0.2792	0.03745	0.1311	0.03643	0.08075	0.1357	0.06408	0.1264
Gg	0.32966	0.04008	0.1322	0.03736	0.09197	0.149	0.06281	0.08191
Dr	0.2599	0.04771	0.1226	0.05293	0.10182	0.1462	0.04869	0.147
Ga	0.3299	0.05002	0.1572	0.04922	0.08588	0.1177	0.04897	0.1405
Xt	0.27894	0.05569	0.1643	0.04505	0.08987	0.1212	0.0643	0.112
Mean	0.2924637	0.0420975	0.1362625	0.04037	0.0924363	0.1318375	0.061008	0.1124763
SD	0.0250758	0.0083937	0.0156789	0.0079384	0.0071074	0.0117254	0.00770647	0.0254739
SEM	0.0088656	0.0029676	0.0055433	0.0028067	0.0025128	0.0041456	0.00272465	0.0090064

Supplementary Table 1. Relative branch lengths from the Maximum Likelihood topology of eIF2α- and other kinases from 8 vertebrate species. The branch lengths from the phylogram depicted in **Figure 1a** of PKR, PERK, HRI, GCN2, WEE1, PBK, NEK2 and TTK from human (Homo sapiens, Hs), cattle (Bos taurus, Bt), mouse (Mus musculus, Mm), rat (Rattus norvegicus, Rn), chicken (Gallus gallus, Gg), frog (Xenopus tropicalis, Xt), stickleback fish (Gasterosteus aculeatus, Ga) and zebrafish (Danio rerio, Dr) are presented, and were used to construct the graph in Figure 1b. Asterisks denote significant difference from PKR ($P<0.001$).

branch	lobe	Sublocation¶	Residue¶¶	TreeSAAP	PAML (BEB)	REL (HyPHY)	tertiary windowing
Vertebrates							
	N	α0	**K261**[a]	***	***	†	yes (261+262)
	C	αD	**E375**	*	**	†	yes (374-376)
	C	αE	**V389**	*	***	†	yes (389-390)
	C	αE	**I405**	***	*	†	yes (405+406)
	C	P+1 loop	**K449**	***	*	†	yes (447-449)
	C	loop (αF-αG)	**D486**	***	*	††	yes (483-490)
	C	αG	**A488**	***	*	††	yes (483-490)
Tetrapods							
	N	loop (α0 – β1)	**I270**	***	*	†	yes (269-272)
	C	β1	**L272**[b]	***	***	†	yes (269-272)
	C	loop (β5-αD)	**F368**	**	*	†	yes (367-369)
	C	αG	**K493**	*	***	†	yes (491-493)
	C	loop (αG - αH)	**D505**	***	*	†	yes (500-506)
	C	loop (αH – αI)	**S520**	***	*	†	yes (518-522)
Mammals + Bird							
	C	β1	**L272**[b]	***	*	†	yes (469-472)
	C	αD	**R382**	*	**	†	yes (382)
	C	β8	**V428**	***	***	†	yes (427-429)
Mammals							
	N/C	loop (αC-β4)	**H322**	*	*	†	yes (322)
	C	αE	**L394**	***	***	†	no
	C	loop (αEF-αF)	**S462**[e]	***	*	††	yes (460-462)
	C	αG	**T491**	*	*	†	yes (491-493)
	C	αG	**D500**	***	*	†	yes (500-506)
	C	αG	**S504**	**	***	†	yes (500-506)
	C	αH	**L514**	***	*	†	yes (513-515)
Primates							
	N	α0	**K261**[a]	***	***	††	yes (261+262)
	N	αC	**K314**	**	***	†	yes (314)
	C	loop (αD-αE)	**K385**[d]	***	***	††	yes (385-387)
	C	P+1 loop	**S448**	**	*	†	yes (447-449)
	C	αH	**L471**	***	*	†	yes (469-472)
	C	loop (αF - αG)	**H483**	***	***	††	yes (483-490)
Rodents							
	C	αD	**I378**	***	*	†	yes (378-381
	C	αD	**E379**	***	*	††	yes (378-381
	C	loop (αEF-αF)	**S462**[e]	n.s.	*	††	yes (460-462)
Chicken							
	C	loop (αD - αE)	**K385**[d]	***	***	†	yes (385-387)
	C	loop (αG - αH)	**I502**	***	*	†	yes (500-506)
Xenopus clade							
	C	β5	**K360**[c]	n.s.	***	††	no
	C	loop (αD - αE)	**K385**[d]	***	*	††	yes (385-387)
	C	loop (αEF-αF)	**S462**[e]	n.s.	*	††	yes (460-462)
	C	αI	**E524**	*	***	†	yes (523-524)
TnPKR3							
	N	α0	**K261**[a]	***	***	††	yes (261+262)
PKZ							
	N	loop (α0 – β1)	**E269**	***	*	†	yes (269-272)
	N	β1	**E271**	***	*	†	yes (269-272)
	N	αC	**R307**	***	*	†	yes (306-310)
	C	β5	**K360**[c]	***	*	††	no

Supplementary Table 2. Residues in the PKR kinase domain under positive selection during evolution.

Residues were identified as under positive selection using the programs TreeSAAP, PAML, REL and tertiary windowing, as indicated. Residues are grouped according to when they were under positive selection; color-coding of the lineages corresponds to Figure 1c,d and Supplementary figure 2. Lobe refers to residues located in the N-terminal (N) and C-terminal (C) lobes of the kinase domain. ¶Sublocations are annotated as in Dar et al. (2005). ¶¶Residues and positions of positively selected residues are shown for human PKR. [a]Residue 261 was positively selected in vertebrate, primate and tetraodon (TnPKR3) lineages, selection in vertebrates is highlighted in Fig. 1. [b]Residue 272 was positively selected in both tetrapods and mammals + bird, selection in tetrapods is highlighted in Fig. 1. [c]Residue 360 was positively selected in PKZ and Xenopus, selection in PKZ is highlighted in Fig. 1. [d]Residue 385 was positively selected in primates, chicken and Xenopus, selection in primates is highlighted in Fig. 1. [e]Residue 462 was positively selected in mammals, rodents and Xenopus, selection in mammals is highlighted in Fig. 1. Asterisks in TreeSAAP and PAML columns denote level of statistical significance [*** = $p<0.001$; ** = $p<0.01$; * = $p<0.05$; n.s. = not significant ($p>0.05$)]. Crosses in the REL column describe sites detected at Bayes Factor 50 (††), and 10 (†) respectively. Tertiary windowing shows the range of adjacent residues under significant positive selection (yes) for a 10 Å window within the protein structure.

Species	Genus	K3L ortholog name (accession #)	E3L ortholog name (accession #)
Vaccinia virus	Orthopoxvirus	K3L (M35027)	E3L (AAA48040)
Variola virus	Orthopoxvirus	C3L (L22579)	E3L (L22579)
Camelpox virus	Orthopoxvirus	CMLV032 (AF438165)	CMLV055 (AF438165)
Cowpox virus	Orthopoxvirus	M3L (X94355)	F3L (X94355)
Rabbitpox virus	Orthopoxvirus	RPXV026 (AY484669)	RPXV048 (AY484669)
Horsepox virus	Orthopoxvirus	HSPV037 (DQ792504)	HSPV060 (DQ792504)
Taterapox virus	Orthopoxvirus	037 (NC_008291)	060 (NC_008291)
Cantagalo orthopoxvirus	Orthopoxvirus	eIF2a-like (AY772447)	E3L (AY771338)
Ectromelia virus	Orthopoxvirus	eIF-2 alpha* (AJ312291)	dsRBD (AJ312294)
Monkeypox virus	Orthopoxvirus	K3L* (AF380138)	MPXVgp052** (AF380138)
Lumpy skin disease virus	Carpipoxvirus	LSDV014 (NC_003027)	LSDV034 (NC_003027)
Goatpox virus	Carpipoxvirus	K3L homolog (AY077835)	E3L homolog (AY077835)
Sheeppox virus	Carpipoxvirus	SPPV_11 (NC_004002)	SPPV_30 (NC_004002)
Myxoma virus	Leporipoxvirus	M156R (NC_001132)	M29L** (NC_001132)
Rabbit fibroma virus	Leporipoxvirus	gp008.2L (NC_001266)	gp029L** (NC_001266)
Yaba monkey tumor virus	Yatapoxvirus	12L (NC_005179)	34L (NC_005179)
Yaba-like disease virus	Yatapoxvirus	12L (NC_002642)	34L (NC_002642)
Tanapox virus	Yatapoxvirus	12L (NC_009888)	34L (NC_009888)
Swinepox virus	Suipoxvirus	SPV010 (C8L) (NC_003389)	SPV032 (NC_003389)
Deerpox virus	unclassified	DpV84gp020 (AY689437)	DpV84gp042 (AY689437)
Bovine papular stomatitis virus	Parapoxvirus	absent	ORF020 (NC_005337)
Orf virus	Parapoxvirus	absent	OV20.0L (AY424975)
Tillquist parapoxvirus	Parapoxvirus	absent	dsRBD (AY278212)

Supplementary Table 3. Poxviruses with orthologs of Vaccinia virus K3L and E3L proteins.
* C-terminal truncation, most likely not functional. ** N-terminal truncation

Residue	found in species	Sublocation	Mutation	found in species	Vaccinia v. K3L-H47R	Variola v. C3L	Variola v. E3L	eIF2αS51A	eIF2B mut.
K261	**Hs, Pt, Ec, Oa**	α0	**S**	**Ma, Tn, Tr, Pp, Ga, Dr, Ssa**	**no effect**	**no effect**	**no effect**	**n. d.**	**n. d.**
K261		α0	**A**	**Cae, Mam, Mm, Ca**	**no effect**	**no effect**	**no effect**	**n. d.**	**n. d.**
K261		α0	**D**	**Rn**	**no effect**	**no effect**	**no effect**	**n. d.**	**n. d.**
E269	**Hs, Pt, Cae, Mam, Cf, Bt, Oc, Xl, Xt**	linker (α0 - β1)	**S**	**Ec, Tn, Tr, Pp, Dr**	**no effect**	**--**	**no effect**	**n. d.**	**n. d.**
L272	**Hs, Pt, Oa, Tn, Tr**	β1	**E**	**Ec, Ma, Mm, Rn, Md, Xt, Dr**	**no effect**	**no effect**	**no effect**	**n. d.**	**n. d.**
N302	**Hs, Pt, Md, Gg, Xt**	linker (β3 – αC)	**S**	**Cae, Mam, Cf, Bt, Oc, Ma, Xl**	**no effect**	**no effect**	**no effect**	**n. d.**	**n. d.**
K360	**Hs, Pt, Cae, Mam, Md, Tn, Tr, Pp, Ga, Dr**	β5	**E**	**Ma, Xl, Xt, Ol,**	**no effect**	**no effect**	**no effect**	**n. d.**	**n. d.**
E375	**Hs, Pt, Cae, Mam, Cf, Ec, Ss, Ma, Mm, Gg, Xl**	αD	**K**	**Xt, Tn, Ol, Ga, Ssa**	**----**	**---**	**+++**	**no effect**	**no effect**
E375			**Q**	**Rn**	**----**	**-**	**++**	**no effect**	**no effect**
I378	**all but Mam, Ma, Mm, Rn**	αD	**M**	**Ma, Mm**	**---**	**-**	**no effect**	**no effect**	**no effect**
E379	**Hs, Pt, Cae, Bt, Oc, Rn, Gg, Tn**	αD	**R**	**Mm, Xt, Tr, Ca**	**---**	**---**	**+++**	**no effect**	**no effect**
R382	**Hs, Pt, Cf, Ec, Bt, Ss, Md, Oa**	αD	**N**	**Mm, Rn, Xl, Xt, Tr, Ol, Pp, Dr, Dr, Ca**	**----**	**----**	**no effect**	**no effect**	**no effect**
K385	**Hs, Pt, Cae, Bt, Oc, Ma, Mm, Xl, Xt**	linker (αD - αE)	**E**	**Mam, Cf, Ec, Ss, Oa, Xt**	**no effect**	**no effect**	**no effect**	**n. d.**	**n. d.**
V389	**Hs, Pt, Cae, Xt, Ca**	αE	**Q**	**Ec, Tn, Tr**	**---**	**---**	**no effect**	**no effect**	**no effect**
S448	**Hs, Pt**	activation loop	**N**	**Cae, Mam, Cf, Ec, Oc, Ma, Md, Gg**	**no effect**	**---**	**no effect**	**n. d.**	**n. d.**
S448			**R**	**Ss, Pp, Dr, Dr, Ca**	**+**	**++**	**++++**	**no effect**	**no effect**
K449	**Hs, Pt, Cf, Bt, Ss, Oc, Oa, Tn, Dr, Dr, Ssa**	activation loop	**T**	**Cae, Mam, Ma, Mm, Rn, Xt, Ol, Pp, Dr**	**no effect**	**no effect**	**no effect**	**n. d.**	**n. d.**
H483	**Hs, Pt, Cae, Bt, Ss, Oc, Ma, Mm, Rn, Oa, Tn**	linker (αF - αG)	**Y**	**Mam, Cf, Ec, Md, Xl, Xt, Pp**	**no effect**	**++**	**+++**	**no effect**	**no effect**
D486	**Hs, Pt, Cae, Mam**	linker (αF - αG)	**N**	**Pp**	**+**	**++**	**++**	**no effect**	**no effect**
D486			**P**	**Cf, Ec, Md**	**-**	**no effect**	**no effect**	**no effect**	**no effect**
A488	**Hs, Pt, Cae, Mam, Ec, Oa, Pp**	αG	**E**	**Mm, Xt**	**+++**	**++++**	**no effect**	**no effect**	**no effect**
K493	**Hs, Pt, Cae, Cf, Bt, Ss, Oc, Ma, Mm,Oa, Gg,Tn, Tr, Pp, Dr, Ssa**	αG	**E**	**Ec, Rn, Xl, Xt, Tr, Dr, Ca**	**no effect**	**---**	**no effect**	**n. d.**	**n. d.**
K493			**Q**	**Mam, Ol**	**no effect**	**--**	**no effect**	**n. d.**	**n. d.**
D500	**Hs, Pt, Cae, Mam, Ma**	αG	**N**	**Bt, Rn, Md, Xl, Xt, Tn, Pp, Dr, Ca**	**no effect**	**no effect**	**---**	**no effect**	**no effect**
D500			**R**	**Oc, Tn, Ol**	**no effect**	**no effect**	**no effect**	**n. d.**	**n. d.**
D505	**Hs, Pt, Cae, Mam, Cf, Bt, Ss, Oc, Ma, Rn**	linker (αF - αG)	**N**	**Ec, Mm**	**no effect**	**no effect**	**no effect**	**n. d.**	**n. d.**

Supplementary Table 4. Summary of mutations in human PKR and yeast growth test assays. The indicated human PKR residues (which are conserved in the listed species and located as annotated in Dar et al. (2005)) were mutated to the indicated amino acids. The mutations were selected based on the presence of the alternate residue in the indicated species. Abbreviations for species are as listed in the legend for **Supplementary Figure 2**. Plasmids expressing WT or the indicated human PKR mutants under the control of a galactose-inducible promoter were introduced into strains expressing vacK3L-H47R, varC3L, varE3L, non-phosphorylatable eIF2α–S51A, or the eIF2Bα-E44D mutant (*gcn3-102* allele). Transformants were streaked for single colonies on galactose medium to induce PKR and K3L (or C3L or E3L) expression and incubated at 30˚C. The growth of colonies was evaluated and is indicated: (-) to (----) for poor growth of yeast relative to transformants expressing WT PKR indicating weak to strong resistance, and (+) to (++++) for better growth than yeast expressing WT PKR indicating weakly to strongly increased sensitivity to vacK3L-H47R, varC3L or varE3L. n.d.= not determined.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
1 Vaccinia virus	***	79.8	87.6	98.9	100	96.6	88.8	98.9	37.1	36	36	25.8	22.5	32.6	33.7	33.7	39.3	39.3
2 Variola virus		***	90.9	81.8	80.7	81.8	92	79.5	33	31.8	33	25	21.6	37.5	36.4	36.4	35.2	39.8
3 Camelpox virus			***	88.8	87.6	88.8	98.9	86.5	33.7	32.6	33.7	22.5	20.2	34.8	33.7	33.7	37.1	38.2
4 Cowpox virus				***	98.9	97.8	89.9	97.8	36	34.8	36	24.7	22.5	32.6	32.6	32.6	39.3	38.2
5 Rabbitpox virus					***	96.6	88.8	98.9	37.1	36	36	25.8	22.5	32.6	33.7	33.7	39.3	39.3
6 Horsepox virus						***	89.9	97.8	36	34.8	36	24.7	22.5	33.7	33.7	33.7	39.3	39.3
7 Taterapox virus							***	87.6	33.7	32.6	33.7	22.5	20.2	36	34.8	34.8	37.1	39.3
8 Cantagalo orthopoxvirus								***	37.1	36	36	25.8	22.5	32.6	33.7	33.7	39.3	39.3
9 Lumpy skin disease virus									***	96.7	96.7	26.7	24.4	35.6	43.3	43.3	46.7	53.3
10 Goatpox virus										***	93.3	26.7	24.4	34.4	42.2	42.2	44.4	51.1
11 Sheeppox virus											***	25.6	25.6	35.6	42.2	42.2	47.8	52.2
12 Myxoma virus												***	54.4	22.3	27.2	27.2	26.2	26.2
13 Rabbit fibroma virus													***	27.8	29.1	29.1	30.4	27.8
14 Yaba monkey tumor virus														***	75.3	75.3	38.2	46.1
15 Yaba-like disease virus															***	98.9	42.7	50.6
16 Tanapox virus																***	42.7	50.6
17 Swinepox virus																	***	44.8
18 Deerpox virus																		***

Supplementary Table 5. Similarity of K3L orthologs in poxviruses
Similarities on the amino acid level are are shown in % as obtained from a multiple alignment using Megalign.

species (common name)	gene	accession number
Bos taurus (cattle)	GCN2	XM_001787661
Bos taurus (cattle)	HRI	NM_001038053
Bos taurus (cattle)	NEK2	NM_001046270
Bos taurus (cattle)	PBK	NM_001102178
Bos taurus (cattle)	PERK	NM_001098086
Bos taurus (cattle)	PKR	AB104655
Bos taurus (cattle)	TTK	XM_603264
Bos taurus (cattle)	WEE1	NM_001101205
Canis familiaris (dog)	PKR	NM_001048135
Carassius auratus (goldfish)	PKZ	AY293929
Chlorocebus aethiops (African green monkey)	PKR	AY623897
Danio rerio (zebrafish)	GCN2	FM210478
Danio rerio (zebrafish)	HRI	NM_001077567
Danio rerio (zebrafish)	NEK2	NM_201050
Danio rerio (zebrafish)	PBK	NM_001002387
Danio rerio (zebrafish)	PERK	XM_695386
Danio rerio (zebrafish)	PKR	AM421526
Danio rerio (zebrafish)	PKZ	AJ852018
Danio rerio (zebrafish)	TTK	NM_175042
Danio rerio (zebrafish)	WEE1	NM_001005770
Equus caballus (horse)	PKR	AY850106
Gallus gallus (chicken)	GCN2	XM_421203
Gallus gallus (chicken)	HRI	NM_204648
Gallus gallus (chicken)	NEK2	NM_001031050
Gallus gallus (chicken)	PBK	XM_420011
Gallus gallus (chicken)	PERK	XM_420868
Gallus gallus (chicken)	PKR	AB125660
Gallus gallus (chicken)	TTK	XM_419867
Gallus gallus (chicken)	WEE1	NM_001031181
Gasterosteus aculeatus (three spined stickleback)	GCN2	ENSGACT00000008173*
Gasterosteus aculeatus (three spined stickleback)	HRI	ENSGACT00000012570*
Gasterosteus aculeatus (three spined stickleback)	NEK2	ENSGACG00000009916*
Gasterosteus aculeatus (three spined stickleback)	PBK	ENSGACG00000006932*
Gasterosteus aculeatus (three spined stickleback)	PERK	ENSGACT00000005852*
Gasterosteus aculeatus (three spined stickleback)	PKR1	AM850085
Gasterosteus aculeatus (three spined stickleback)	TTK	ENSGACG00000005223*
Gasterosteus aculeatus (three spined stickleback)	WEE1	ENSGACT00000012920*
Homo sapiens (human)	GCN2	NM_001013703
Homo sapiens (human)	HRI	NM_001134335
Homo sapiens (human)	NEK2	NM_002497
Homo sapiens (human)	PBK	NM_018492
Homo sapiens (human)	PERK	AF193339
Homo sapiens (human)	PKA	NM_207518
Homo sapiens (human)	PKR	NM_002759
Homo sapiens (human)	TTK	NM_003318
Homo sapiens (human)	WEE1	NM_003390
Macaca mulatta (Rhesus macaque)	PKR	EF467667

species (common name)	gene	accession number
Mesocricetus auratus (golden hamster)	PKR	DQ645944
Monodelphis domestica (gray short-tailed opossum)	PKR	ENSMODT00000030492*
Mus musculus (house mouse)	GCN2	NM_013719
Mus musculus (house mouse)	HRI	NM_013557
Mus musculus (house mouse)	NEK2	AF007247
Mus musculus (house mouse)	PBK	NM_023209
Mus musculus (house mouse)	PERK	AF076681
Mus musculus (house mouse)	PKR	NM_011163
Mus musculus (house mouse)	TTK	NM_009445
Mus musculus (house mouse)	WEE1	NM_009516
Oryctolagus cuniculus (European rabbit)	PKR	DQ115394
Oryzias latipes (Japanese medaka)	PKR	AM850088
Pan troglodytes (chimpanzee)	PKR	XM_001166391
Pimephales promelas (fathead minnow)	PKR	AM850089
Rattus norvegicus (Norway rat)	GCN2	XM_230462
Rattus norvegicus (Norway rat)	HRI	NM_013223
Rattus norvegicus (Norway rat)	NEK2	AF352021
Rattus norvegicus (Norway rat)	PBK	XM_224300
Rattus norvegicus (Norway rat)	PERK	NM_031599
Rattus norvegicus (Norway rat)	PKR	NM_019335
Rattus norvegicus (Norway rat)	TTK	XM_236477
Rattus norvegicus (Norway rat)	WEE1	NM_001012742
Salmo salar (Atlantic salmon)	PKZ	ABA64562
Sus scrofa (pig)	PKR	NM_214319
Takifugu rubripes (torafugu)	PKR1	AM850086
Takifugu rubripes (torafugu)	PKR2	AM850087
Tetraodon nigroviridis (green spotted pufferfish)	PKR1	AM421523
Tetraodon nigroviridis (green spotted pufferfish)	PKR2	AM421524
Tetraodon nigroviridis (green spotted pufferfish)	PKR3	AM421525
Xenopus laevis (African clawed frog)	PKR1	AM421528
Xenopus tropicalis (western clawed frog)	GCN2	FM210530
Xenopus tropicalis (western clawed frog)	HRI	NM_001045573
Xenopus tropicalis (western clawed frog)	NEK2	BC075559
Xenopus tropicalis (western clawed frog)	PBK	NM_001011346
Xenopus tropicalis (western clawed frog)	PERK	FM210531
Xenopus tropicalis (western clawed frog)	PKR1	AM850090
Xenopus tropicalis (western clawed frog)	PKR2	AM850091
Xenopus tropicalis (western clawed frog)	PKR3	AM850092
Xenopus tropicalis (western clawed frog)	TTK	ENSXETG00000011768*
Xenopus tropicalis (western clawed frog)	WEE1	NM_001126965

Supplementary Table 6. Accession numbers of genes used for phylogenetic analyses.
* Ensembl identification numbers are shown