

Figure S1. **Contrasting selective pressures on eIF2 α kinases, eIF2 α , and K3L.**

- (a) A diagram illustrates the activity of eIF2 α kinases (PKR, HRI, PERK and GCN2), which block protein synthesis. Ribbon representations of the kinase domain of PKR, eIF2 α , and K3L are shown (PDB codes 2A1A and 1LUZ)^{15,39}. dN/dS calculations from human and rhesus sequences suggest that PKR is under positive selection, while the other eIF2 α kinases and eIF2 α appear to be under purifying selection. K3L appears to be under positive selection in a comparison of vaccinia and smallpox virus sequences.
- (b) Sliding window analysis of dN/dS for human and rhesus sequence comparisons of PKR and PERK. Sliding windows of 350bp every 50bp of PKR suggest strong signatures of positive selection with a notable peak mapping to the kinase domain. In contrast, sliding windows of 500bp every 50bp of PERK suggest signatures of purifying selection with no data points reaching a dN/dS value of 1.
- (c) Viral antagonists reported to directly interact with PKR are shown according to the localization of interacting regions in PKR¹⁰. Other viral factors, including vIRF-2 (human herpes virus 8) and SM (Epstein-barr virus), have also been reported to directly interact with PKR¹⁰. Residues highlighted by likelihood ratio tests as being subject to positive selection with high posterior probabilities ($p > 0.90$) are indicated by red arrowheads from an analysis of 21 variants of PKR from primates.

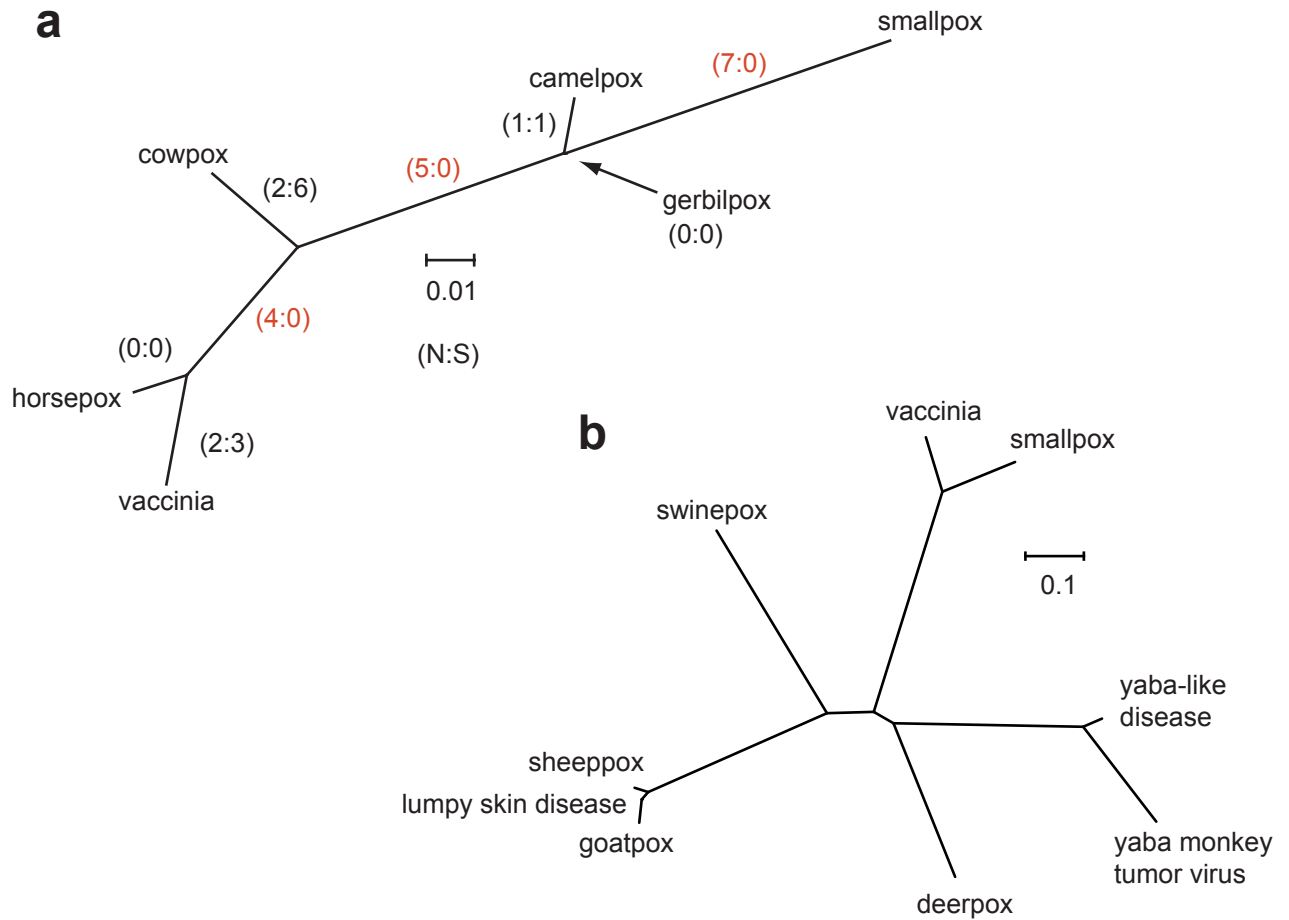


Figure S2. **Evolutionary analysis of K3L in poxviruses.**

- (a) A neighbor-joining phylogenetic tree of K3L amino acid sequences from orthopox genomes. Ratios of non-synonymous (N) to synonymous (S) substitutions as calculated by PAML free-ratio analysis are shown for each branch.
- (b) A neighbor-joining tree of K3L amino acid sequences from a variety of poxviruses. Note the scale differences of evolutionary distance between (a) and (b).

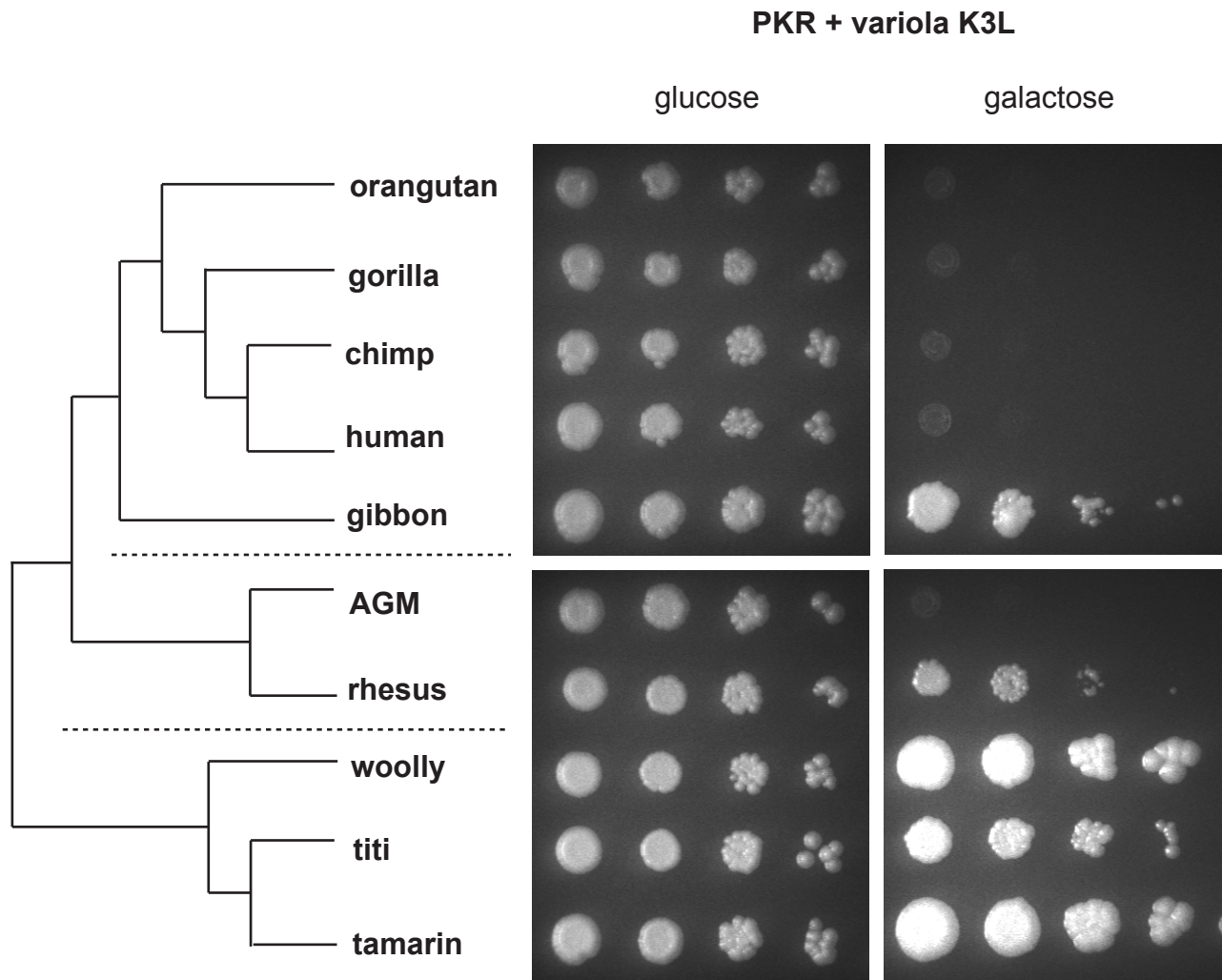


Figure S3. **Rescue of PKR induced growth arrest by variola virus K3L.**

Plasmids encoding PKR variants from a panel of primates under pGal were introduced into yeast strain HM4 (eIF2 α and K3L-variola). Ten-fold serial dilutions of transformants were spotted on plates containing either glucose or galactose. These results are qualitatively identical to those obtained with vaccinia K3L (Figure 1c).

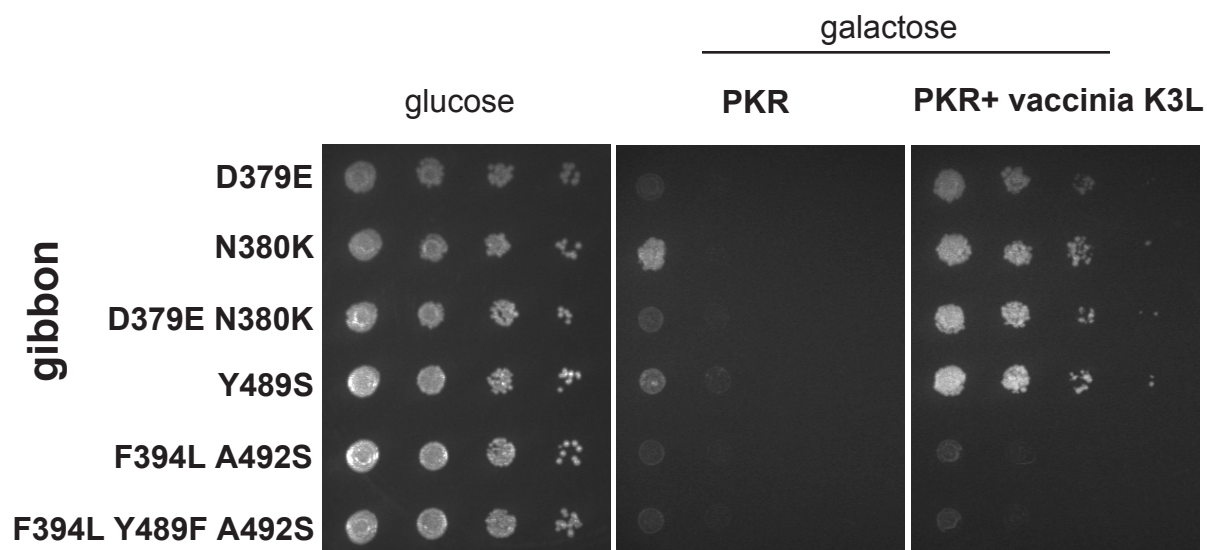


Figure S4. **Substitutions in the α D, α E, and α G helices of gibbon PKR.**

Ten-fold serial dilutions of transformants expressing alleles of gibbon PKR with combinations of substitutions in the α D (379, 380), α E (394), and α G (489, 492) helices are shown.

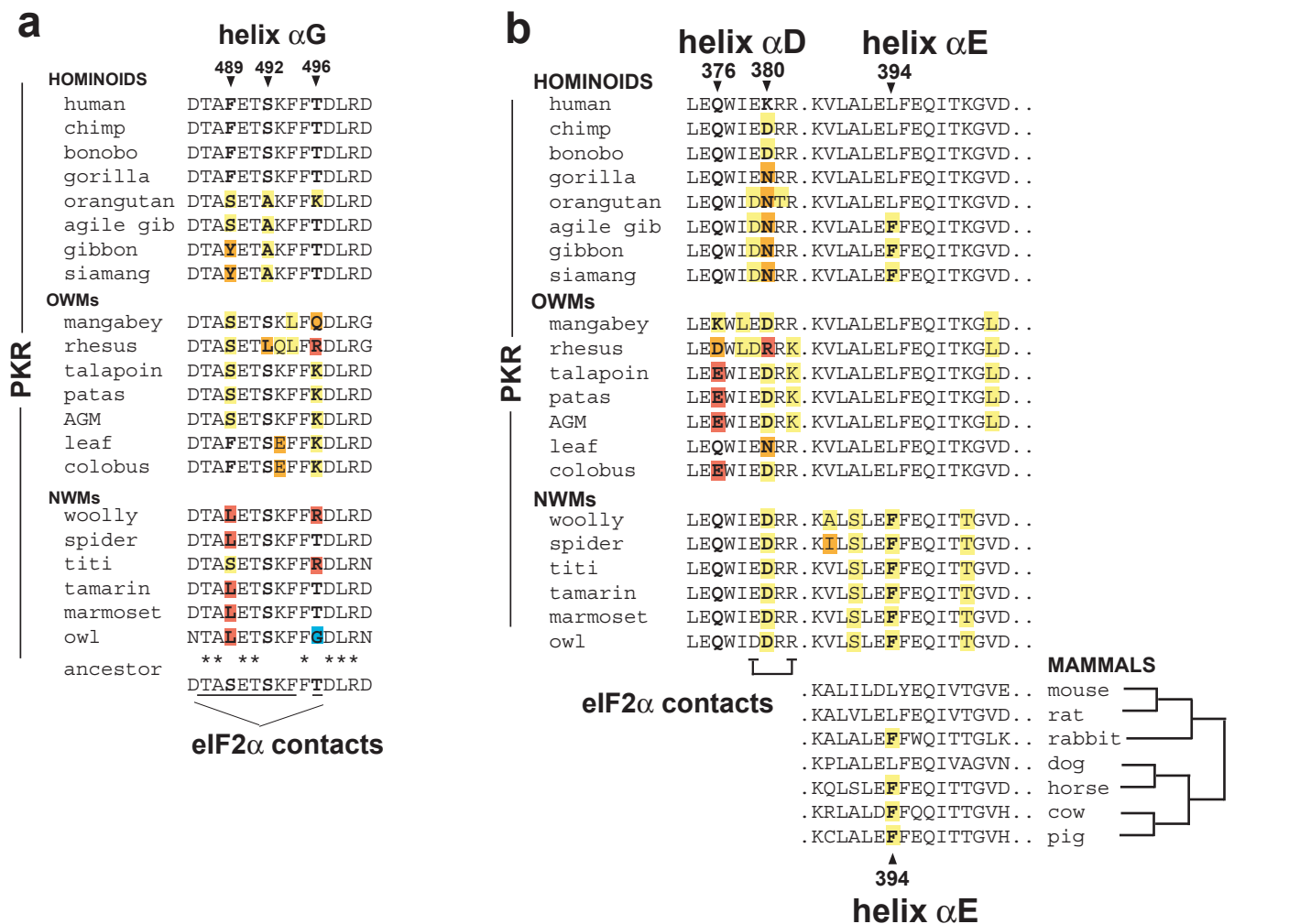


Figure S6. **Amino acid alignments of the α G, α D, and α E helices of PKR.**

- (a) An alignment of the α G helix from a simian primate panel highlighting residues under positive selection (black arrowheads). The frequency of substitutions at each position is indicated by a color code (yellow for a single substitution, orange for a second substitution, and red for a third substitution, blue for a fourth substitution) with regard to human PKR. eIF2 α contacts are also noted.
- (b) A sequence alignment of helices α D and α E highlighting residues under positive selection and position 394 (black arrowheads). Changes are color-coded according to substitution frequencies. For the alignment of the α E helix, sequence from an additional set of mammals is shown, which highlights toggling between Leu and Phe at position 394 across the mammalian lineage. eIF2 α contacts in helix α D are also noted.

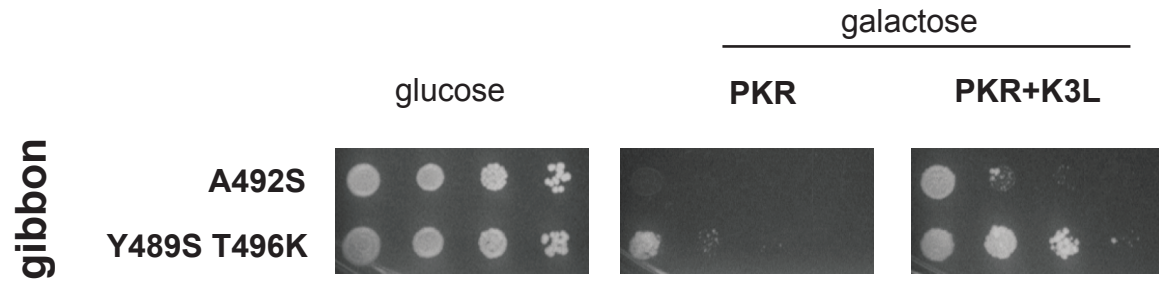


Figure S7. **Substitutions in the α G helix of gibbon PKR.**

Ten-fold serial dilutions of transformants expressing substitutions in the α G helix of gibbon PKR. Y489S/T496K is a gibbon variant with the α G helix from orangutan that is compromised for growth inhibition (middle panel), suggesting a reduction in recognition of eIF2 α . Wildtype orangutan PKR recognizes eIF2 α and resists K3L (Figure 1c).

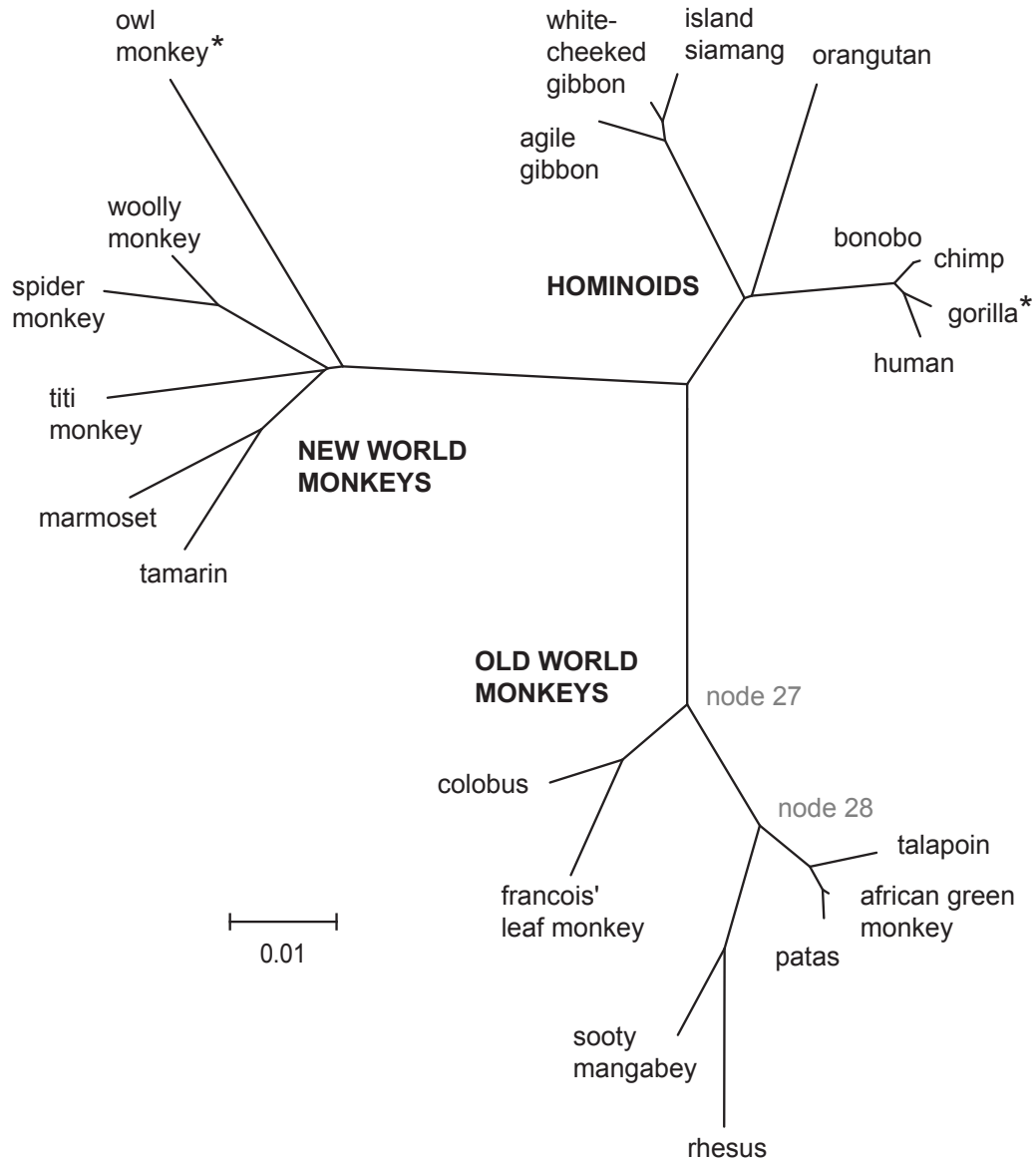


Figure S8. **Phylogenetic tree of primate PKR.**

A neighbor-joining tree based on PKR sequence from hominoids, Old and New World monkeys. Asterisks by gorilla and owl monkey indicate branches where species appear differently than on the generally accepted primate phylogeny (Figure 1a; also see Full Methods). The nodes highlighted in the Old World monkey clade refer to points on the tree where reconstructed sequences were compared in the Supplementary data. This branch has a 22:0 ratio of non-synonymous (N) to synonymous (S) changes (Figure 1a).