

## Supplementary Information

### Activation of Protein Kinase PKR Requires Dimerization-induced *cis*-phosphorylation within the Activation Loop

Madhusudan Dey, Brian Rick Mann, Ashish Anshu, M. Amin-ul Mannan

Department of Biological Sciences  
University of Wisconsin-Milwaukee  
Milwaukee, WI-53211

To whom correspondence should be addressed: Madhusudan Dey, Department of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI-53211, Room 460, Lapham Hall, 3209 N Maryland Avenue, Phone: 414-229-4309, Fax: 414-229-3926, E-mail: [deym@umw.edu](mailto:deym@umw.edu)

**Figure S1: The PKR-KD bound to eIF2 $\alpha$  superimposes well on the PKR-KD<sup>K296R</sup>**

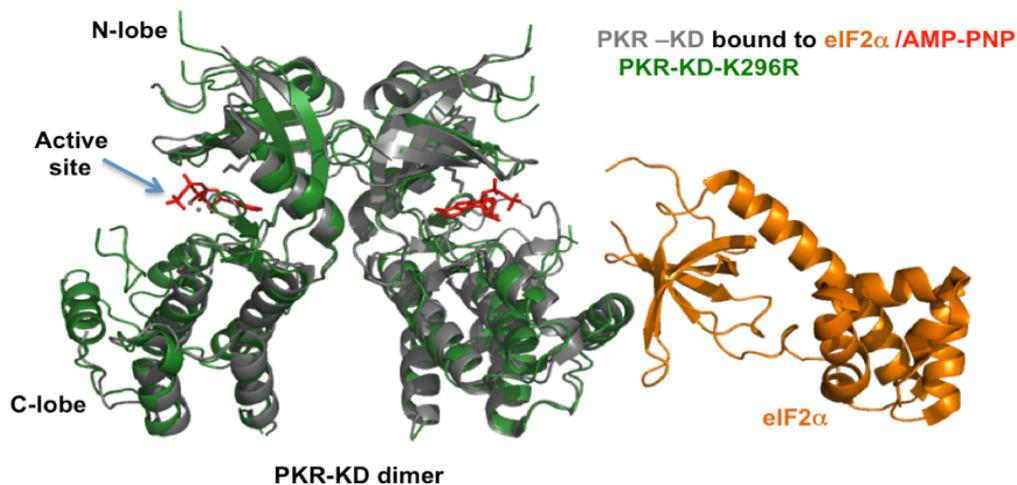


Fig S1: Structural coordinates of the PKR-KD-K296R (PDB ID: 3UIU) and the PKR-KD bound to eIF2 $\alpha$  and AMP-PNP (PDB ID: 2A19) [1] were aligned in a single PyMol file by pair wise alignment (root mean square deviation = 1.508). Proteins are shown as a ribbon-presentation (PKR-KD in grey; PKR-KD-K296R in green; eIF2 $\alpha$  in orange; and AMP-PNP in red). Each kinase domain composed of a smaller N-terminal lobe (N-lobe) and larger C-terminal lobe (C-lobe).

**Table 1: List of plasmids used in this study**

Plasmid	Description	Reference
D109	Flag-tagged-PKR-WT	[2]
D110	Flag-tagged PKR-K296R	[2]
D112	Flag-tagged PKR-D266R	[2]

D771	Flag-tagged PKR-Y323A	[2]
D119	Flag-tagged PKR-T446A	[2]
D111	Flag-tagged PKR-T446D	This study
D113	Flag-tagged PKR-R307A	This study
D114	Flag-tagged PKR-R307K	This study
D116	Flag-tagged PKR-K304A	This study
D122	Flag-tagged PKR <sup>phk1</sup>	This study
D123	Flag-tagged PKR <sup>phk1</sup> -E182A	This study
D607	Flag-tagged PKR <sup>phk1</sup> -D266R	This study
D771	Flag-tagged PKR <sup>phk1</sup> -Y323A	This study
D121	Flag-tagged PKR <sup>Chk1</sup>	This study
D930	GST-PKR-KD in Trp vector	This study
D935	LIM-PKR-K296H	This study
D934	LIM-PKR-D414A	This study
D911	LIM-PKR-K296R	This study
D936	LIM-PKR <sup>phk1</sup> -KD	This study
D845	GST-eIF2 $\alpha$ (1-180)	[3]
P722	GCN2	[4]
pC901	LIM-PKR-KD	[3]
pC903	Ldb-PKR-KD	[3]
p1079	pEMBlyx4::URA3	Vector plasmid
p2444	pEMBlyx4::Trp	Vector plasmid
p180	GCN4 <i>LacZ</i> reporter plasmid	[4]

#### Reference:

1. Dar, A.C., T.E. Dever, and F. Sicheri, *Higher-order substrate recognition of eIF2 $\alpha$  by the RNA-dependent protein kinase PKR*. Cell, 2005. **122**(6): p. 887-900.
2. Dey, M., et al., *Mechanistic link between PKR dimerization, autophosphorylation, and eIF2 $\alpha$  substrate recognition*. Cell, 2005. **122**(6): p. 901-13.
3. Ung, T.L., et al., *Heterologous dimerization domains functionally substitute for the double-stranded RNA binding domains of the kinase PKR*. EMBO J, 2001. **20**(14): p. 3728-37.
4. Mueller, P.P. and A.G. Hinnebusch, *Multiple upstream AUG codons mediate translational control of GCN4*. Cell, 1986. **45**(2): p. 201-7.