Supplementary Information

1. Materials and Methods

Immunoprecipitation and immunoblotting

Protein extraction, immunoblotting and immunoprecipitation were performed as previously described (Su *et al.*, 2006). Fifty μg and 1 mg of protein from whole cell extracts (WCE) were used for immunoblotting and immunopreciptation respectively. The following antibodies were used: Anti-phosphotyrosine 4G10 mouse Ab (Upstate Biotechnology Inc); Anti-phospho-Jak1 pY1022/1023 rabbit Ab (Cell Signaling); anti-human PKR mouse monoclonal F9 antibody (Li and Koromilas, 2001); anti-mouse PKR mouse Ab (B-10; Santa Cruz); anti-eIF2α rabbit Ab (Cell Signaling); anti-eIF2αpS51 rabbit Ab (Li *et al.*, 2001); anti-FLAG (M2) mAb (Sigma); anti-Jak1 mouse mAb (BD Transduction Laboratories); anti-Tyk2 rabbit Ab (Santa Cruz); anti-PKRpT446 polyclonal Ab (Upstate Biotechnology Inc); anti-VSV-G mAb (Roche); anti-PKRpY101 Ab and anti-PKRpY293 rabbit Ab (Su *et al.*, 2006). Quantification of the bands in the linear range of exposure was performed by densitometry using the NIH Image 1.54 software.

Transient transfections and pull down assays.

For the transient expression of Jak1 and Flag-PKR (Fig. 3B), U4A cells were infected with recombinant vaccinia virus containing the T7/RNA polymerase gene (Fuerst *et al.*, 1986) for 1 h followed by transfection with 1 μg of pMT2T plasmid DNA bearing either WT or a kinase dead (KD) K896R mutant of Jak1 cDNA and 1 μg pcDNA3/zeo plasmid containing the catalytically inactive Flag-PKRK296R cDNA in the presence of Lipofectamine Plus reagent (Invitrogen) for 16 h.

Supplementary Figure legends

Supplementary Figure 1. (**A**) PKR interaction with Tyk2 *in vitro*. VSV-G-Tyk2 was transiently expressed in HeLa cells (Li *et al.*, 1999) and protein extracts (500 μg) were incubated with either glutathione-sepharose beads alone (lane 2) or equal amounts (100 ng) of bead-bound GST alone (lane 3), GST-N-PKR (aa 1-262; lane 4), GST-C-PKR (aa 263-551; lane 5) and full length (FL) GST-PKR (aa 1-551; lane 6). Bound proteins were detected by immunoblotting with an anti-VSV-G mAb. The amount of VSV-G-Tyk2 in 50 μg of protein extracts (10% input) is indicated in lane 1. (**B**) Mapping of Tyk2 interaction domains with PKR. The schematics represent the N-terminus truncated VSV-G-Tyk2 proteins which were transiently expressed in HeLa cells and protein extracts (500 μg) were incubated with 100 ng of bead-bound GST-N-PKR (panel a, lanes 2, 4, 6, and 8) or 100 ng of GST-C-PKR (panel b, lanes 2, 4, 6, and 8). Bound proteins were then subjected to immunoblotting with an anti-VSV-G mAb (top and bottom panels). Protein extracts (50 μg) containing the VSV-G-Tyk2 truncated proteins used for the interaction (10% input) are shown in lanes 1, 3, 5, and 7. The structure domains featured in Tyk2 are referred to as the Jak homology regions (JH1-JH7). (**C**) Histidine-tagged Tyk2 proteins truncated from the N- or C-terminus were expressed and purified from bacteria (shown in the schematic). Fusion proteins were incubated with extracts expressing Flag-tagged N-PKR. Bound proteins were detected by immunoblotting with anti-Flag Abs (panel a) or stained with Coomassie Blue to visualize His-Tyk2 fusion proteins (panel b). As a negative control, activated nickel resin was incubated with Flag-N-PKR extracts (lane 9). Ten % input of Flag-N-PKR extracts were used as control (lane 1).

Supplementary Figure 2. Verification of the specificity of the interaction between Jaks and PKR. 2fTGH or U4A cells were treated as in Figure 2C for the indicated time(s). Protein extracts (1 mg) were subjected to immunoprecipitation with anti-PKR monoclonal Ab (lane 1) or control anti-mouse IgG Ab (lanes 2-4) followed by immunoblotting with the indicated antibodies. The protein level of Jak1 was detected by immunoblotting of 50 μg of protein from whole cell extract (WCE; panel c).

Supplementary Figure 3. 2fTGH and U4A cells were mock transfected (lanes 1,4) or transfected with 10 μg/ml dsRNA (lanes 2, 3, 5, 6) for the indicated times (Baltzis *et al.*, 2002). Protein extracts (50 μg) were subjected to mmunoblot analysis for eIF2α phosphorylated at serine 51 (panel a) followed by immunoblotting for total eIF2α (panel b).

References

1 Baltzis D, Li S, and Koromilas AE (2002) Functional characterization of pkr gene products expressed in cells from mice with a targeted deletion of the N terminus or C terminus domain of PKR. *J Biol Chem,* **277:** 38364-38372

2 Li S, Labrecque S, Gauzzi MC, Cuddihy AR, Wong AH, Pellegrini S, Matlashewski GJ, and Koromilas AE (1999) The human papilloma virus (HPV)-18 E6 oncoprotein physically associates with Tyk2 and impairs Jak-STAT activation by interferon-alpha. *Oncogene,* **18:** 5727-5737