Supplemental figures

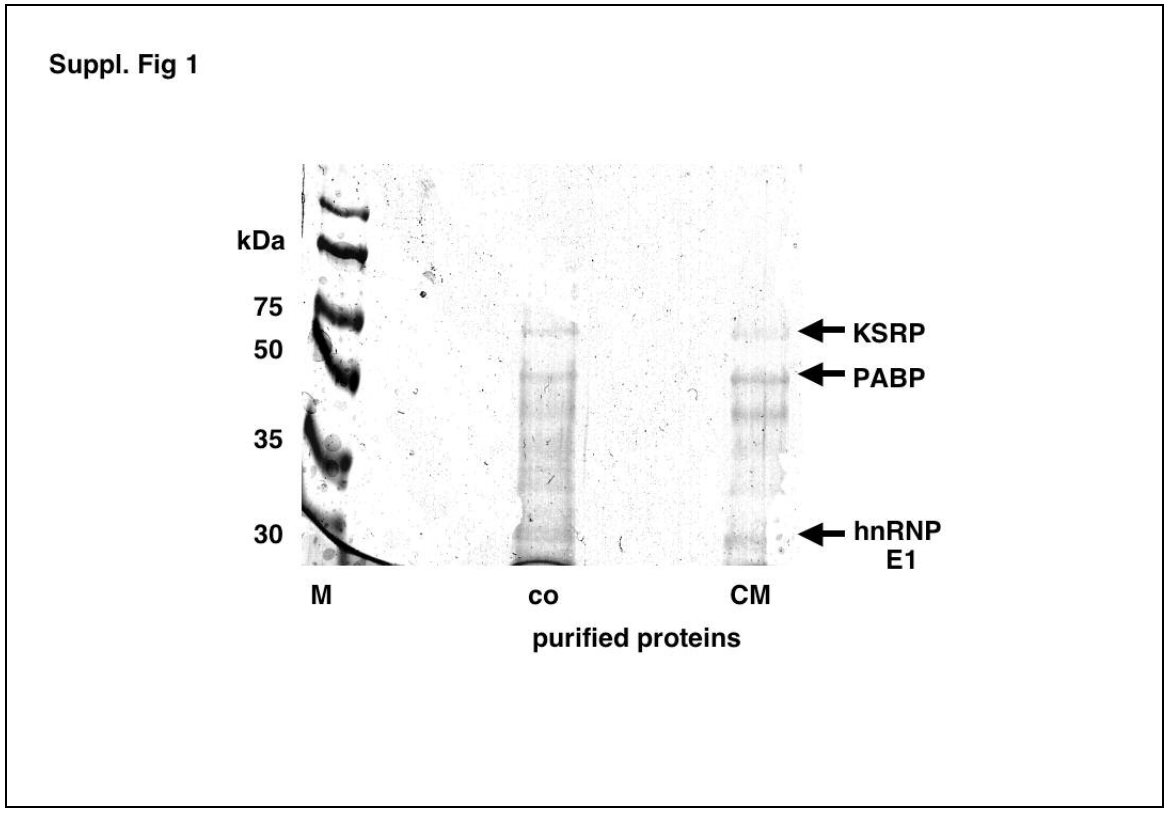

Figure 1. Purification of RNA binding proteins interacting with the 3'-UTR of the human iNOS mRNA

To purify proteins binding to the 3'-UTR of the human iNOS mRNA affinity chromatographies using biotinylated iNOS-3'-UTR-RNA were performed (1,2) as described in Material and Methods. DLD-1 cells were preincubated for 18h in medium without FCS and phenol red. Then cells were incubated with (**CM**) or without (**Co**) the cytokine mixture for 6h and protein extracts were isolated. These extracts were incubated with biotinylated iNOS-3'-UTR-RNA and streptavidine-agarose beads. After several washing and centrifugation steps the RNA-binding proteins were eluted by 2 M KCl.

A representative colloidal coomassie blue-stained SDS PAGE used to separate the affinity purified RNA binding proteins is shown. The stained protein bands were cut out and the identity of the proteins was determined by peptide mass fingerprinting (Toplab, München, Germany). The names of the identified proteins are indicated (**M**: molecular weight standard; **KSRP**, KH-type splicing regulatory protein; **PABP1**, polyadenylate-binding protein 1; **hnRNP E1**, heteronuclear ribonucleoprotein E1).

1. Grosset, C., Chen, C.Y., Xu, N., Sonenberg, N., Jacquemin-Sablon, H. and Shyu, A.B. (2000) *Cell*, **103,** 29-40.
2. Bollig, F., Winzen, R., Gaestel, M., Kostka, S., Resch, K. and Holtmann, H. (2003) *Biochem Biophys Res Commun*, **301,** 665-670.

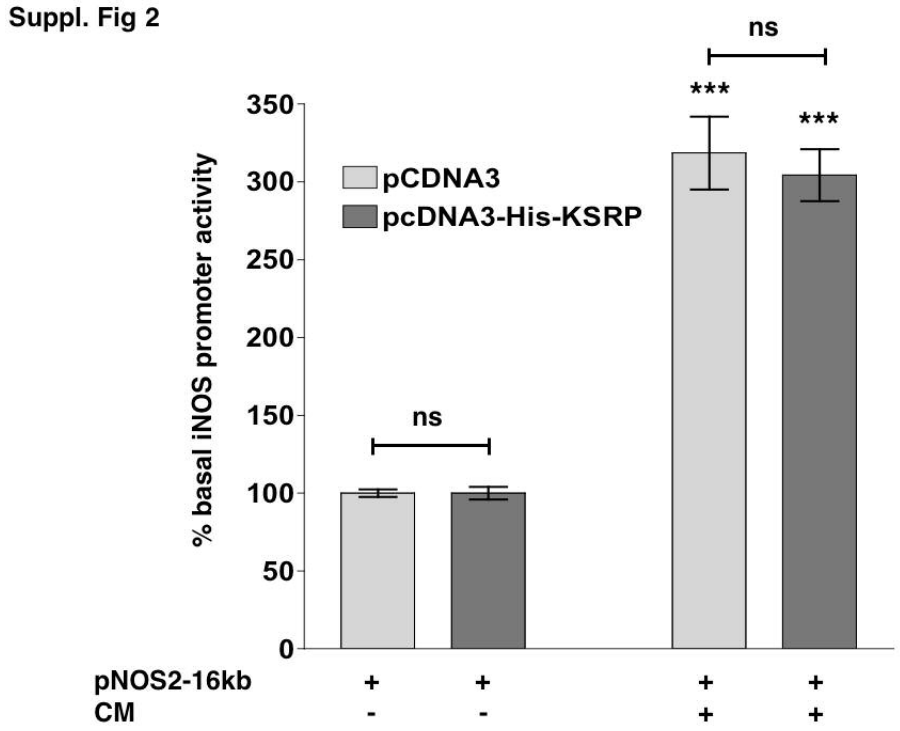

Figure 2. No effect of overexpression of KSRP on human iNOS promoter activity
DLD-1 cells were transfected with a firefly luciferase reporter gene under the control of the 16 kb human iNOS promoter (pNOS2-16kb), a renilla luciferase expression plasmid (for normalization of transfection efficiency) and either pcDNA3 or pcDNA3-His-KSRP. After transfection, cells were incubated for 24h with medium containing FCS. Then cells were incubated for 16h with medium without FCS. Then cells were further incubated with or without a mixture of cytokines (**CM**) for 6h. The cells were lyzed with Passive Lysis Buffer (Promega, Heidelberg, Germany) and firefly luciferase and renilla luciferase activity were determined using the Dual Luciferase Assay system (Promega, Heidelberg, Germany). Values for firefly luciferase were divided by the values for the renilla luciferase. Columns (mean ± SEM) represent the cytokine-induced activation of the iNOS promoter expressed as a percentage of the corresponding basal values determined in the absence of cytokines (*** $p < 0.001$ vs control cells; ns = not significant vs pcDNA3-treated cells).