Supplemental Experimental Procedures

Primers for cloning and mutagenesis: The primers are displayed in Table 1. The primers used for cloning *PIG3* cDNA into pGEX-4T-2 and pCMV-HA contain restriction sites for *Eco*RI, in the forward primer, and *Sal*I in the reverse primer.

RNA-Electrophoretic Mobility Shift Assay (EMSA)—In vitro transcription and EMSA were performed as previously described (1). Purified protein (10-500 ng) was incubated at room temperature for 10 min, in a 10 µl-reaction mixture containing 10 mM Hepes, pH 7.4, 25 mM potassium acetate, 2.5 mM magnesium acetate, 0.5 µg yeast tRNA, 0.5% Igepal CA 630, 5% glycerol, 1 mM dithiothreitol, and 10 units of RNAsin. Subsequently, 25 fmol of labeled RNA probe were added. Samples were incubated for 20 min, at room temperature, and then subjected to electrophoresis in a 5% polyacrylamide gel, at 170 V, for 90 min, using 90 mM Tris, 110 mM boric acid, and 2 mM EDTA, pH 8.5, as a running buffer. After electrophoresis, gels were incubated for 20 min in a 20% (v/v) ethanol and 10% (v/v) acetic acid aqueous solution, introduced into plastic bags and imaged using a PhosphorImager screen (BioRad).

Supplemental Results

Interaction of PIG3 with RNA—We have recently demonstrated that human and yeast ζ-crystallins bind AU-rich elements in RNA (1). Using the EMSA technique, we investigated whether PIG3 showed a similar interaction. As indicated in Fig. 1, PIG3 did not bind $A(UUUA)_5$, an RNA sequence which is recognized by all AU-rich element binding proteins including ζ-crystallins. No binding was either found with the probe derived from the pBluescript vector (result not shown).

Supplemental References

1. Fernández, M. R., Porté, S., Crosas, E., Barberà, N., Farrés, J., Biosca, J. A., and Parés, X. (2007) *Cell Mol Life Sci* **64**, 1419-1427

Supplemental Table S1. Primers for cloning and mutagenesis		
PIG3		
pGEX-4T-2 & pCMV-HA	F	5’-GGTATTGGAATTCCCATGTTAGCCGTGCAC-3’
	R	5’-AGAGGAGAGTTGTCGACCTACTGGGGCAGTTCC-3’
pET-30 Xa/LIC	F	5’-GGTATTGAGGGTCGCATGTTAGCCGTGCAC-3’
	R	5’-AGAGGAGAGTTAGAGCCCTACTGGGGCAGTTCC-3’
Tyr51Phe	F (45-56)	5′-GCAGAGACAAGGCCAGT**T**TGACCCACCTCCAGGAGCC-3′
	R (44-55)	5′-CCTGGAGGTGGGTCA**A**ACTGGCCTTGTCTCTGCATTAAG-3′
Tyr51Ala	F (45-56)	5′-GCAGAGACAAGGCCAG**GC**TGACCCACCTCCAGGAGCC-3′
	R (44-55)	5′-CCTGGAGGTGGGTCA**CG**CTGGCCTTGTCTCTGCATTAAG-3′
Ser151Val	F (145-158)	5’-CTAATCCATGCAGGACTG**GT**TGGTGTGGGCACAGCTGCTATC-3’
	R (143-156)	5’-GCTGTGCCCACACCA**AC**CAGTCCTGCATGGATTAGCACATAG-3’
ζ-crystallin		
Tyr59Phe	F (54-66)	5’-CATTCGCTCTGGTACTT**T**TAGTAGAAAACCACTCTTACCC-3’
	R (52-64)	5’-AGTGGTTTTCTACTA**A**AAGTACCAGAGCGAATGTATGTCT-3’
Tyr59Ala	F (54-66)	5’-CATTCGCTCTGGTACT**GT**TAGTAGAAAACCACTCTTACCC-3’
	R (52-64)	5’-AGTGGTTTTCTACTA**GC**AGTACCAGAGCGAATGTATGTCT-3’

F (Forward primer). R (Reverse primer). In primers used for mutagenesis, the amino acid positions that correspond to primers are displayed in brackets. Mutated nucleotides are underlined.

Supplemental Figure S1

Figure S1 Binding analysis of $A(UUUA)_5$ with Zta1p (yeast ζ-crystallin) and PIG3 using EMSA. RNA sequences containing $A(UUUA)_5$ were labeled with ^{32}P-UTP and incubated with increasing amounts of purified PIG3. Zta1p (100 ng) was used as a positive control of interaction. Positions of free probe (f) and shifted complex (c) are indicated.