SUPPLEMENTARY DATA

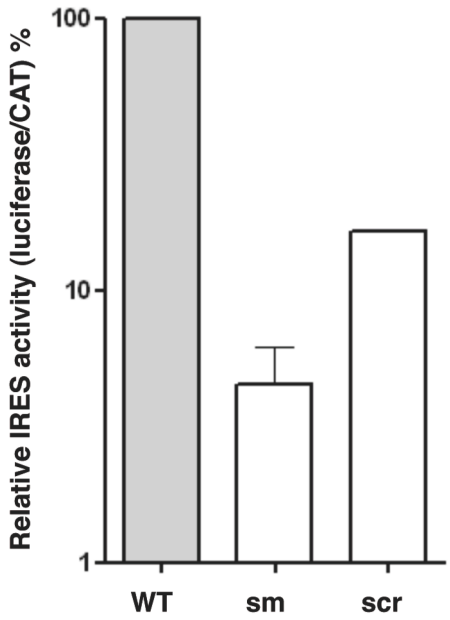

Figure S1. Relative activity of IRES elements bearing substitutions that enhance the binding of Gemin5. Relative activity of IRES elements harboring the sm (A_{416}AUAGGUG$_{423}$ to AAU**UUU**UG) or scr (A_{416}AUAGGUG$_{423}$ to A**C**CA**C**GUG) substitutions (bold letters) on the left of domain 5 hairpin was determined in BHK-21 cells transfected with plasmids of the form CAT-IRES-luciferase as the ratio of luciferase to CAT made relative to the activity obtained with the wt IRES. Values correspond to the mean of three independent assays. Errors bars, s.d.

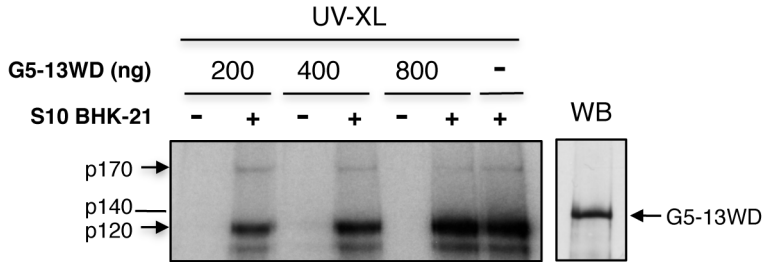

Figure S2. The N-terminal region of Gemin5 is unable to photocrosslink to IRES RNA. UV-crosslinking assay conducted with increasing amounts (0 to 800 ng) of purified His-tagged G5-13WD and radiolabeled domain 5, in the presence (+) or absence (-) of a cytoplasmic S10 BHK-21 cellular extracts (20 µg). Arrows mark the position of Gemin5 (p170) and the expected position of G5-13WD (p140). The mobility of G5-13WD detected by western blot using anti-Xpress is shown on the right.

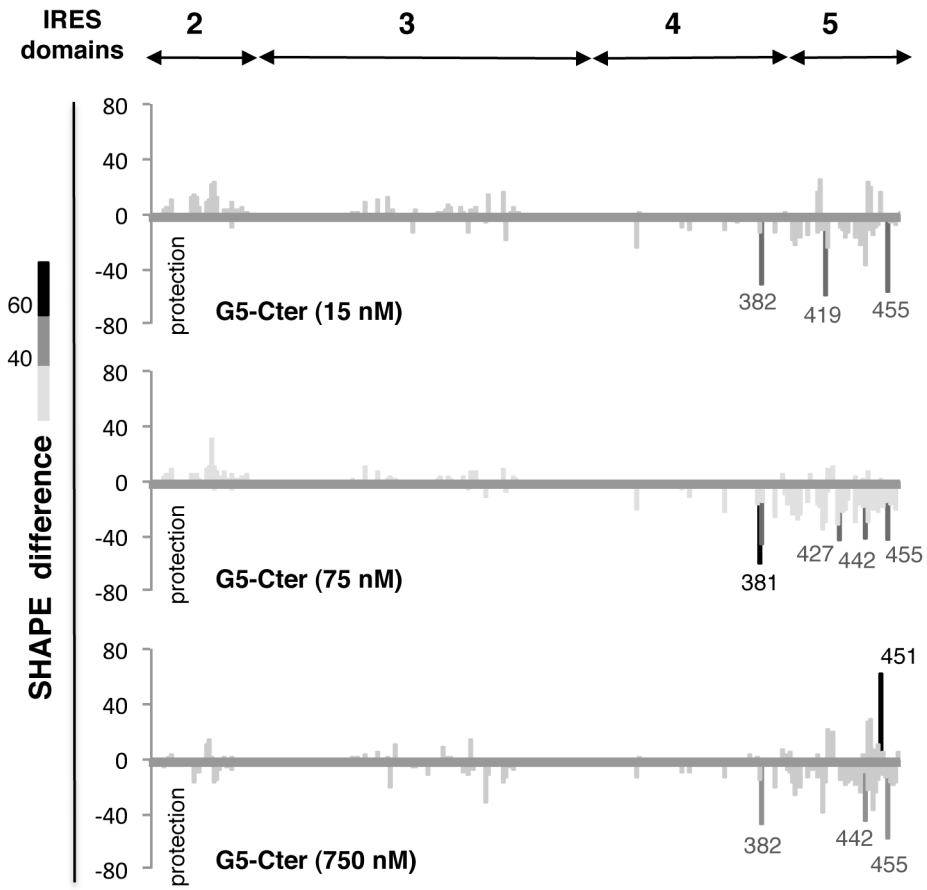

Figure S3. Modification of RNA SHAPE reactivity by the C-terminal region of Gemin5. SHAPE difference plots of the IRES RNA incubated with G5-Cter (15, 75 or 750 nM) relative to naked RNA. Nucleotides with absolute changes in SHAPE reactivity greater than 60 are depicted in black, while those between 40 and 60 are marked in dark grey.

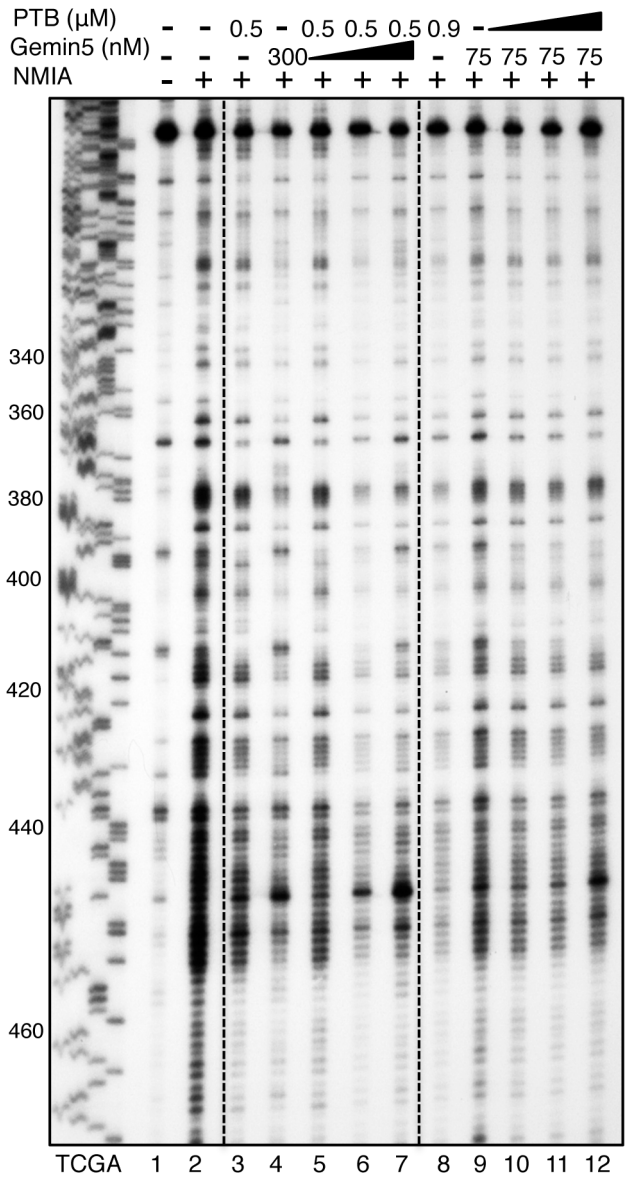

Figure S4. Effect of Gemin5 on PTB-assisted IRES folding. Primer extension analysis of RNA-protein complexes, assembled with the indicated concentrations of Gemin5 and PTB, treated (+) or untreated (-) with NMIA, conducted with a 5′-end labeled primer. Increasing concentrations of Gemin5 (10, 75, 300 nM) was used to assemble RNA-protein complexes with PTB (0.5 μM), while increasing concentrations of PTB (10, 500, 900 nM) was used to assemble RNA-protein complexes with Gemin5 (75 nM). Nucleotide positions are indicated on the left according to the sequencing lanes (TCGA) obtained with the same labeled primer; cDNA full-length products are shown at the top of each lane.

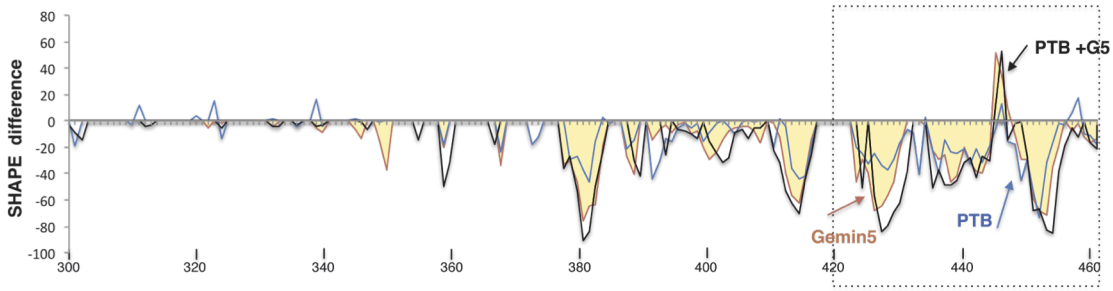

Figure S5. Modification of the difference in SHAPE reactivity induced upon addition of Gemin5 and PTB. SHAPE difference profiles of the IRES incubated with Gemin5 (300 nM), PTB (500 nM), alone or combined, relative to free RNA.

Table S1. Oligonucleotide sequences

Primer	Nucleotide sequence (5'-3')
sG5.1EcoRI	GGGGAATTCTGGTGGTCTCTC
asG5EcoRI	CGGGAATTCATGACCGGTACGCGTA
sG5.2Eco	CGCGAATTCATGTGGTGGTCTCTG
asG5SalI	GCCGTCGACCCTTCATACAG
asd5IIrest	GTAAAGGAA**GAA**TGCCGAC
sd5IIbase	CTTCTAGAATTCAAT**GAA**TGACCGG
asd5II	GAGGTCGACTCTAGAGGATCCTC
sd5stem	TCTAGAATTCAATAGGTGACGCCAGGTC
sd5IIWT	CTTCTAGAATTCAATAGGTGAC
asd5II5U/A	AATTGT**TTT**GG**TT**AGGTGCCGAC
sd5II5U/A	CT**AA**CC**AAA**ACAATTAATGAC
sd5IIsm	GCGCGAATTCAAT**TTT**TGACCGGAG
sd5IIscr	CCTGAATTCA**CC**A**C**GTGACCG
sId5-sm	CGGTTTAAAAAGCTTCTACGCCTGAAT**TTT**TGACCGGAGGTCGG
asId5-sm	CCGACCTCCGGTCA**AAA**ATTCAGGCGTAGAAGCTTTTTAAACCG
sId5-scr	AAGCTTCTACGCCTGA**CC**A**C**GTGACCGGAGGTCGGC
asId5-scr	GCCGACCTCCGGTCAC**G**T**GG**TCAGGCGTAGAAGCTT