SUPPLEMENTARY INFORMATION

Figure S1. HSQC spectrum of a 1H-15N-labelled Gemin5-np domain (blue) in 50 mM Phosphate buffer with 150 mM NaCl, pH 6.7, acquired in a 600 MHz spectrometer. About ~80% of the signals corresponding to the domain are defined in the spectrum, precluding the unambiguous assignment of all resonances. However, based on the assignment of the sequence using standard backbone triple resonance experiments and on the dispersion of the NMR signals shown in the spectrum, we conclude that the construct investigated has a short helical conformation surrounded by unstructured regions, yielding the ensemble of flexible conformations observed in 2D and 3D NOESY experiments. The presence of these elements of secondary structure is in agreement with the secondary structure prediction, which it is represented in Figure 2B.

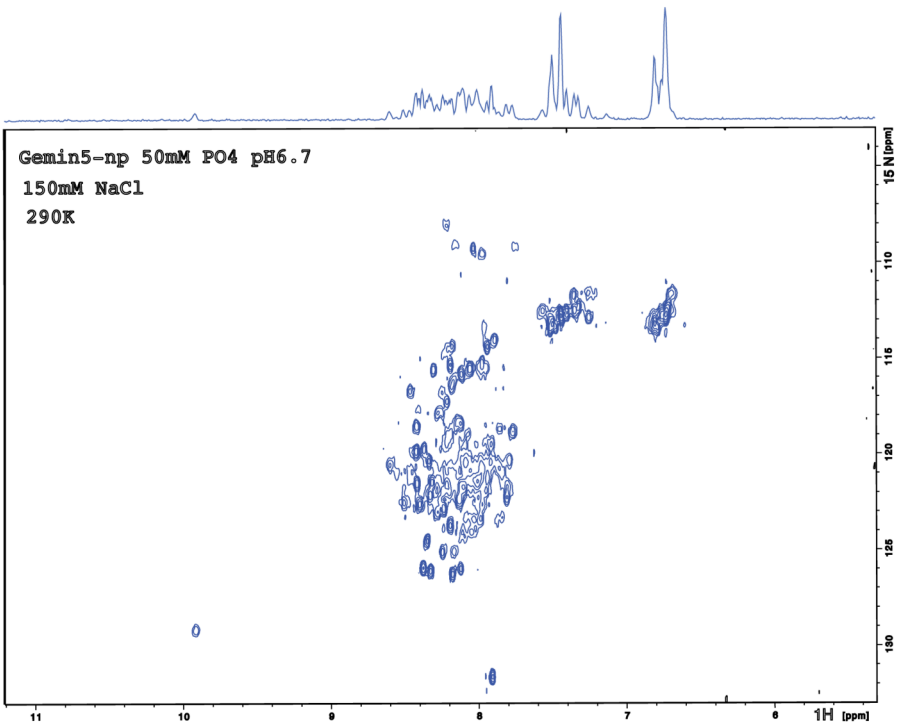

Figure S2. Depletion of Gemin5 in HEK293 cells. (A) Western blot analysis of HEK293 cell extracts transfected with increasing concentrations (25 to 100 nM) of three different siRNAs (G5.1, G2.2 and G5.3) targeted to Gemin5 or a control siRNA with no target sequence in mammalian mRNAs. Cell extracts were collected 48 and 72 h post-transfection. Tubulin was used as loading control. (B) The effectiveness of depletion for each G5 siRNA (dark grey, light grey, or white shaded bars), relative to the control siRNA (black bars), is represented in a histogram. (C) The effect on luciferase expression following transfection of a bicistronic construct in siG5.3 transfected cells is shown on the bottom panel.

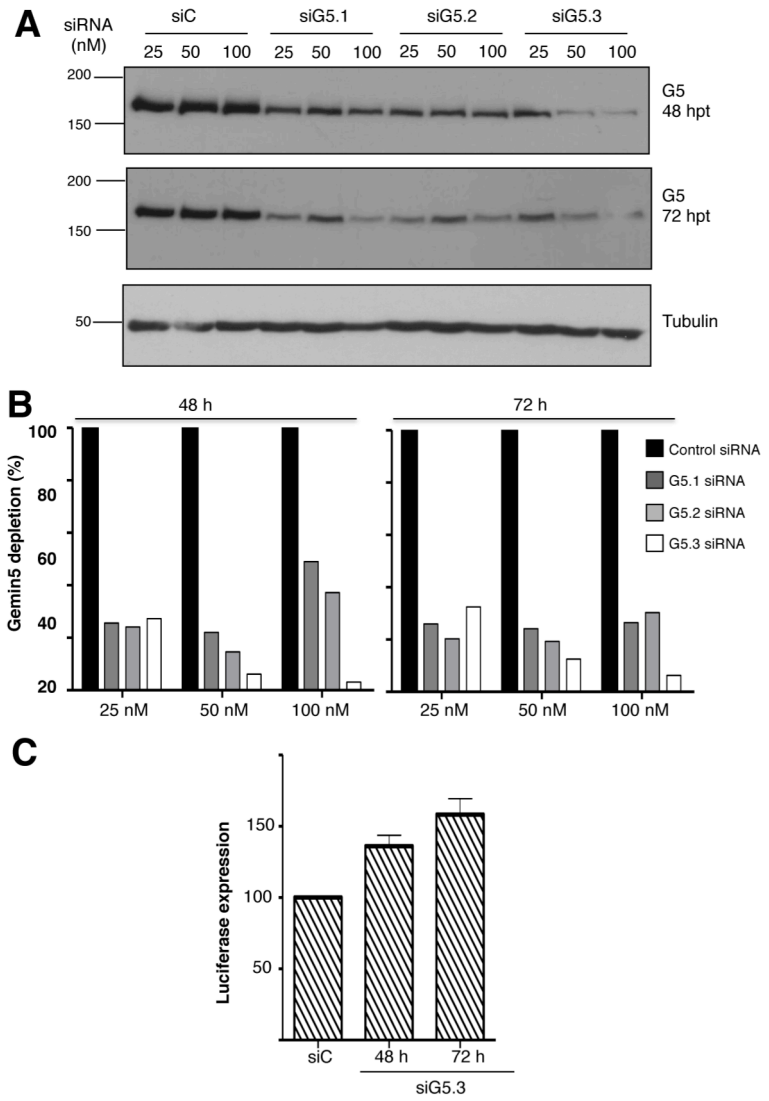

Figure S3. Effect of Gemin5 truncated polypeptides expression on cap-dependent translation. G5-depleted cells were transfected with increasing amounts of the indicated G5 truncated constructs and the bicistronic pBIC. Cap-dependent translation was monitor by CAT activity, relative to the values observed in control siRNA treated cells (empty bars). Black bars depict the effect of Gemin5 silencing on cap-dependent translation, while striped bars depict the values of CAT activity observed in G5 constructs transfected cells.

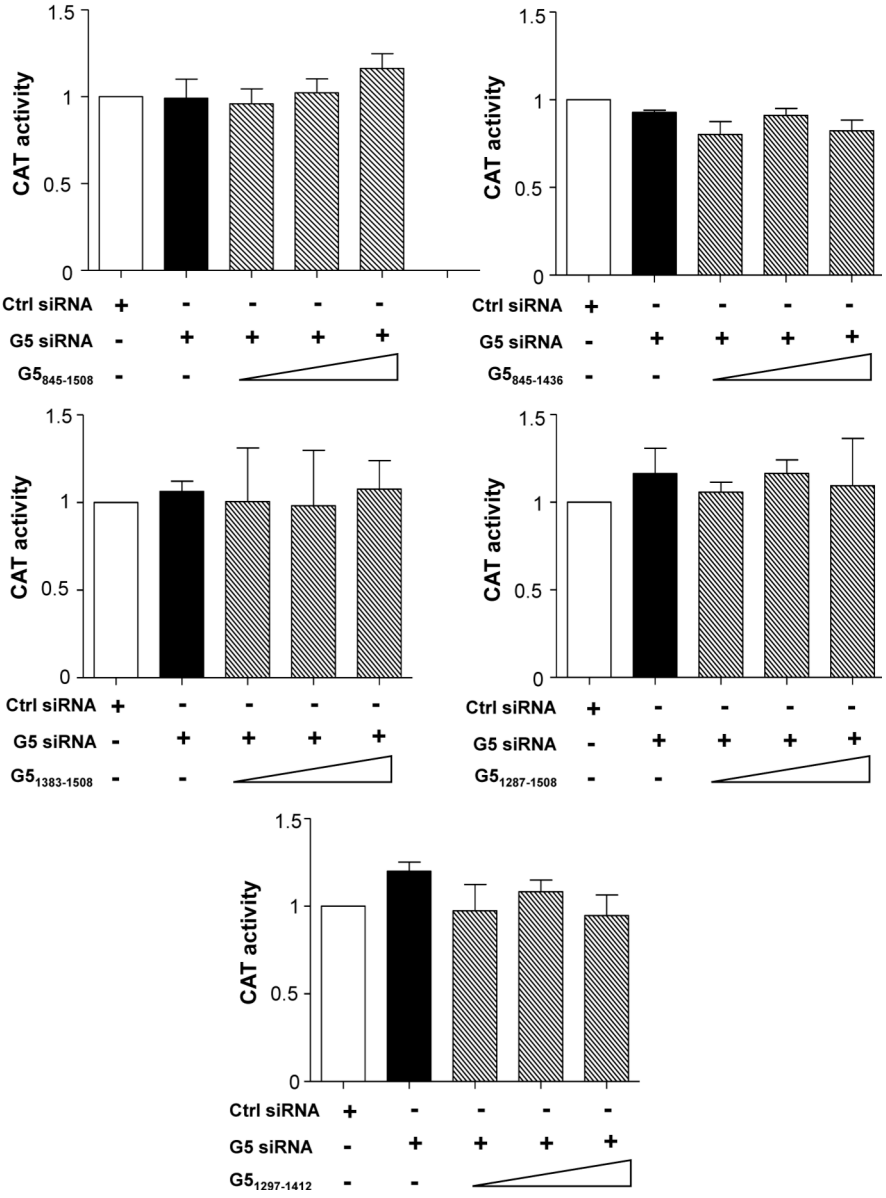

Figure S4. The C-terminal region of Gemin5 represses IRES-dependent translation in vitro. Construct $pG5_{1383-1508}$ was created with primers J1s/15as using as template $pG5_{1287-1508}$ and inserted into pGEM3 via KpnI-XbaI. Transcript $G5_{1383-1508}$ (0.1–5 pmol) synthesized *in vitro* was translated in 70% rabbit reticulocyte lysate (RRL) (Promega) supplemented with ^{35}S-methionine (10 μCi) 15 min prior to addition of the bicistronic RNA (200 ng) bearing the FMDV IRES between CAT and luciferase. ^{35}S-labeled proteins were resolved in SDS-PAGE followed by autoradiography of dry gels. The intensity of ^{35}S-labeled luciferase (squares) (IRES-dependent translation) and chloramphenicol acetyl transferase (CAT) (triangles) (5´-end dependent translation) proteins was measured in a densitometer. Values represent the mean (±SD) of three experiments normalized to the lane without G5 RNA.

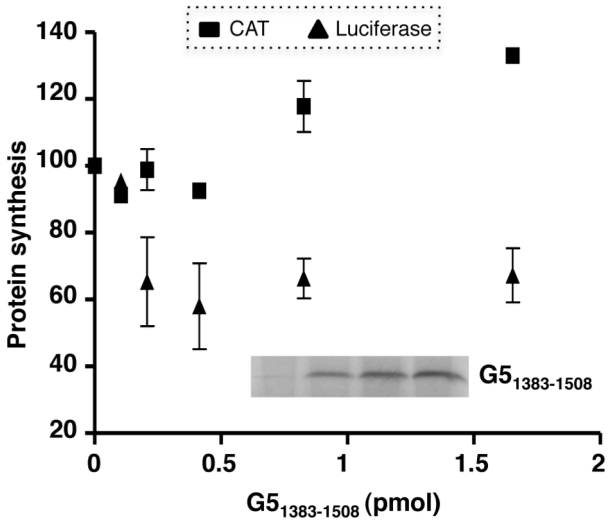

Figure S5. UV-crosslinking assay conducted with increasing amounts of the deletion His-tagged $G5_{\Delta 1365-1394}$ and radiolabeled domain 5, fractionated in SDS-PAGE and visualized by autoradiography. The mobility of the protein detected by WB using anti-Gemin5 of the purified protein is shown on the right. Mobility of MW markers is indicated at the left.

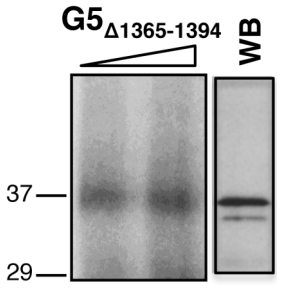

Table S1. Oligonucleotides

Primer	Nucleotide sequence (5'-3')	Construct
G5-1s	GGATCCCGCTCGTTCCTTG	pcDNAXpressG5$_{845-1508}$
G5-2as	CCACCAGAATTCATACAGAC	pcDNAXpressG5$_{845-1508}$
G5-3s	GCTTGGTACCAATGATCCGACAACACC	pcDNAXpressG5$_{1383-1508}$
G5-4as	CTCTAGAGGATCCCCGGGTCACATAC	pcDNAXpressG5$_{1383-1508}$
G5-5s	GGATCCCTGGTGGTCTCTCTC	pcDNA3XpressG5$_{1287-1508}$
G5-6as	TCTAGATCACATACAGAAGGTCTG	pcDNA3XpressG5$_{1287-1508}$
G5-7s	GGTACCGCCAAATTCCAGTGTCTGGG	pcDNA3Xpress-G5$_{1297-1412}$
G5-8as	TTACTGCTCTGCTTCTACTTCC	pcDNA3Xpress-G5$_{1297-1412}$
G5-9s	CATATGGCTCGTTCCTTGCTTCCCC	pET28aG5$_{845-1436}$
G5-10as	CCACCAGAATTCATACAGAC	pET28aG5$_{845-1436}$
G5-11s	TGAATTCTGGTGGTCTCTCTC	pET28aG5$_{845-1436}$
G5-12as	GCTAAGCTTCATTTGGTTAACTCAGG	pET28aG5$_{845-1436}$
G5-13s	GGGGAATTCTGGTGGTCTCTC	pET28aG5$_{1287-1508}$
G5-14as	TTGCTCGAGTCACATACAGAAGGTCTGGCAGTG	pET-28aG5$_{1287-1508}$
G5-15s	CGCATGCACTCTGTAAATCCAC	pRSETB-G5$_{\Delta 1365-1394}$
G5-16as	GAAGCTTGATGATGACCGGTAC	pRSETB-G5$_{\Delta 1365-1394}$
G5-17s	AAGGTACCATGCGGGGTTCTCATC	pYES2-G5$_{845-1508}$
G5-18as	AAGCGGCCGCTCACATACAGAAGGTCTGGC	pYES2-G5$_{845-1508}$
G5-19s	GGCGGTACCATGATCCGACAACACC	pG5$_{1383-1508}$
G5-20as	GAACGCGGCTACAATTAATAC	pG5$_{1383-1508}$