SUPPLEMENTARY DATA

The RNA-binding protein Gemin5 binds directly to the ribosome and regulates global translation

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SUPPLEMENTARY FIGURES

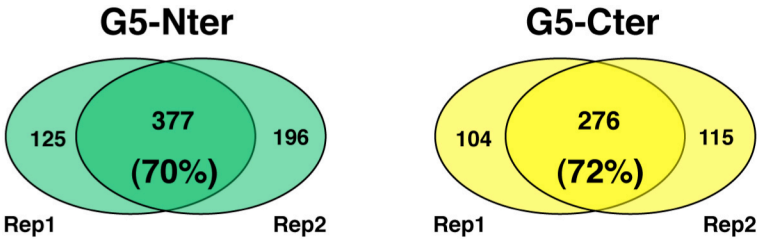

Figure S1. Venn diagram showing the overlap between proteins identified in independent biological replicates. Green and yellow ovals depict proteins associated to G5-Nter and G5-Cter, respectively. Numbers indicate the absolute number of proteins identified in the replicate assays (Rep1 and Rep2) of each protein.

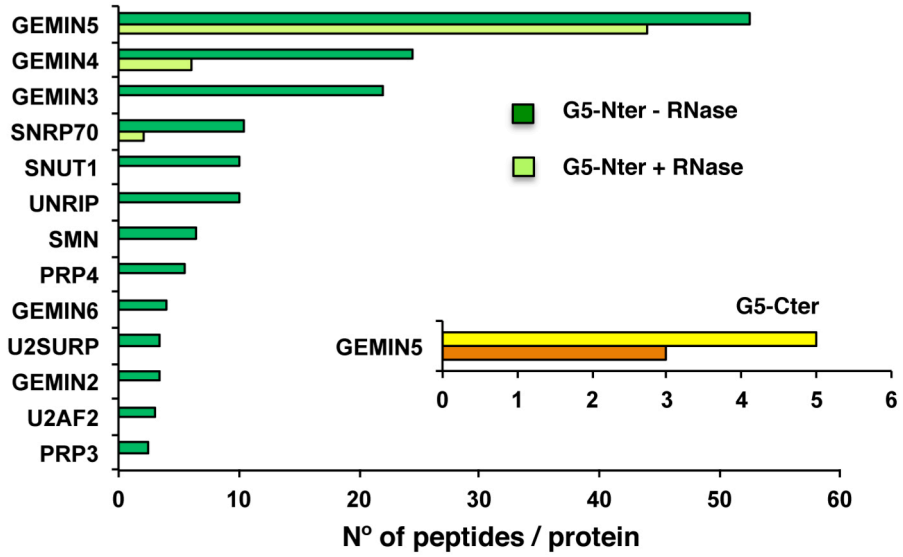

Figure S2. Members of the SMN complex and related proteins differentially associated to Gemin5-TAP fragments. Dark green and yellow bars depict proteins associated to G5-Nter and G5-Cter, respectively. Light green and orange bars represent SMN members resistant to RNase treatment during the purification process, associated to G5-Nter or G5-Cter, respectively. The plot represents the average of peptides identified in the replicate assay of each protein.

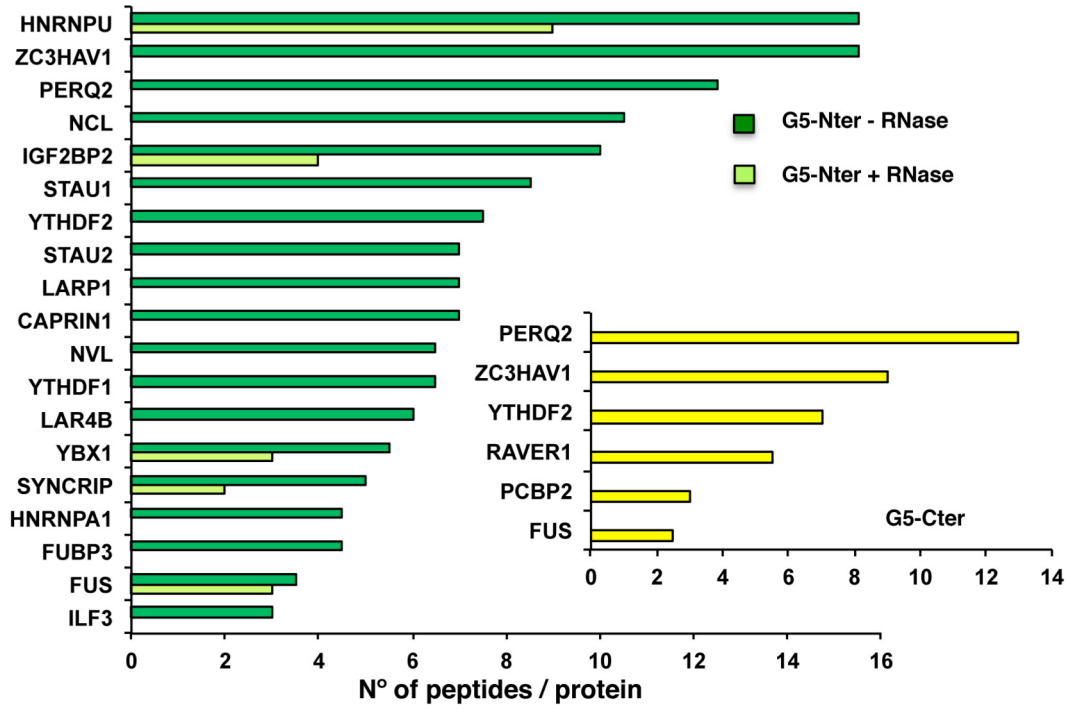

Figure S3. IRES-transacting factors differentially associated to G5-Nter or G5-Cter in the absence or presence of RNase treatment during the purification process. Colors are used as in Figure S2.

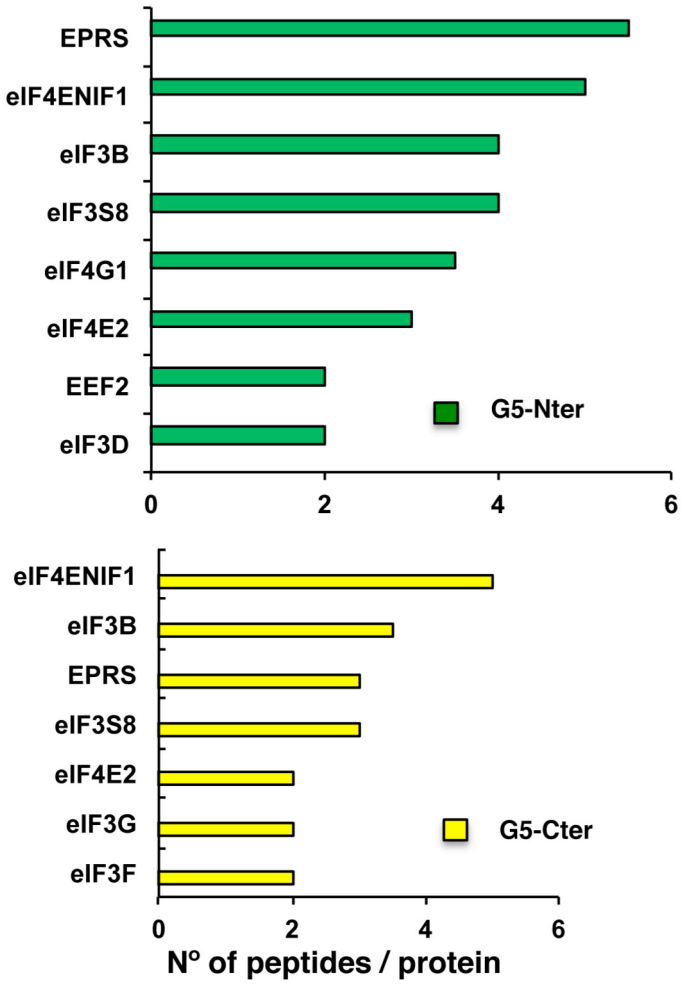

Figure S4. Factors involved in protein synthesis, differentially associated to G5-Nter or G5-Cter in the absence or presence of RNase treatment during the purification process. Colors are used as in Figure S2.

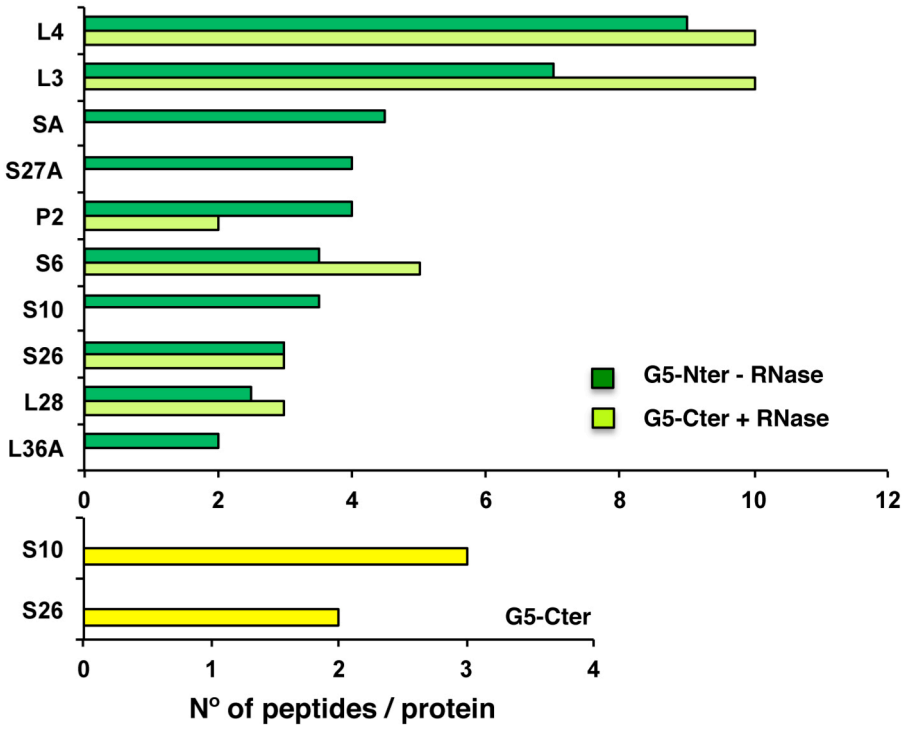

Figure S5. Ribosomal proteins differentially associated to G5-Nter or G5-Cter in the absence or presence of RNase treatment during the purification process. Colors are used as in Figure S2.

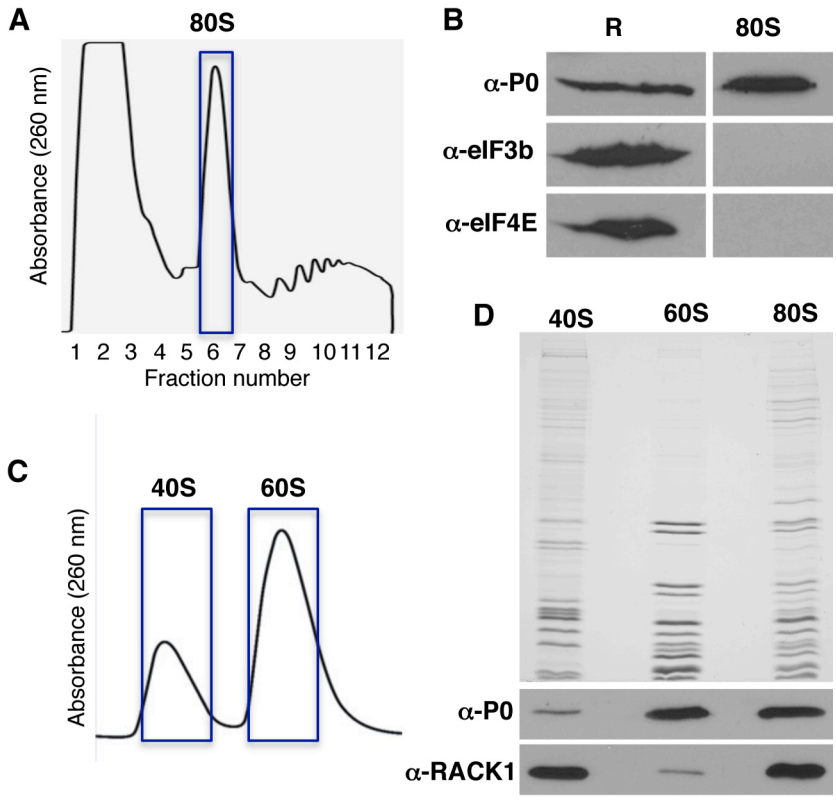

Figure S6. 80S ribosome, 40S and 60S subunits preparation for pull-down assays. (A) 80S ribosomes were prepared by centrifugation of the fractions corresponding to the 80S peak separated on a 10-50% sucrose gradient. (**B)** Absence of associated factors in the 80S ribosomes sediment (80S) was determined by WB against P0, eIF3b, eIF4E (right panel); R corresponds to the ribosome fraction, prepared as in Figure 3A. (**C)** Dissociation gradient showing the 40S and 60S peaks used to prepare 40S and 60S subunits. (D) Coomassie blue stained SDS-PAGE loaded with 40S, 60S and 80S samples used in the binding assays. The purity of these samples was analyzed by WB against P0 (60S) and Rack1 (40S).

Table S1. Oligonucleotides

5´Xpress-G5-EcoRI	5’ TGAATTCTGGTGGTCTCTCTC 3’
3´Xpress-G5-XbaI	5’ TCTAGATCACATACAGAAGGTCTG 3’
5’NTAP-G5-13WD-PacI	5’ CATTAATTAACATGGGGCAGGAGC 3’
3’NTAP-G5-13WD-NotI	5’ AAGCGGCCGCTTATTCATACAGACGCCCAT 3’
5’CTAP-G5CT-NotI	5’ AAGCGGCCGCACCATGGAATTCTGGTGGTCTCTC 3’
3’CTAP-G5CT-PacI	5’ CATTAATTAACATACAGAAGGTCTGGC 3’
G5_F381As	5’ CCTTCCCTTGGTGGGGCTGCATACAGCCTGGC 3’
G5_F381Aas	5’ GCCAGGCTGTATGCAGCCCCACCAAGGGAAGG 3’
5'EcoRI-GST-HNRNPU	5’ ATGAATTCCCATGAGTTCCTCGC 3’
3'SalI-GST-HNRNPU	5’ ATGTCGACTCAATAATATCCTTGGTGATAATG 3’
5'EcoRI-GST-IGF2BP2	5’ ATGAATTCTGATGAACAAGCTTTACATC 3’
3'SalI-GST-IGF2BP2	5’ ATGTCGACTCACTTGCTGCGCT 3’
5'SmaI-GST-SYNCRIP	5’ ATCCCGGGCATGGCTACAGAACATG 3’
3'XhoI-GST-SYNCRIP	5’ ATCTCGAGTCATTGTAACAGGTCAGG 3’
5'EcoRI-GST-RPS3A	5’ AAGAATTCAAATGGCGGTTGGC 3’
3'SalI-GST-RPS3A	5’ ATGTCGACTTAAACAGATTCTTGGACT 3’
5'SmaI-GST-RACK1	5’ ATCCCGGGAATGACTGAGCAGATGAC 3’
3'SalI-GST-RACK1	5’ ATGTCGACTCAGCGTGTGCCAAT 3’
5'SmaI-GST-RPL3	5’ ATCCCGGGTATGTCTCACAGAAAGTTCT 3’
3'EcoRI-GST-RPL3	5’ ATGAATTCTTAAGCTCCTTCTTCCTTTG 3’
5'BamHI-GST-RPL4	5’ AAGGATCCATGGCGTGTGCTC 3’
3'SalI-GST-RPL4	5’ ATGTCGACTTATGCAGCAGGCTTC 3’
5'EcoRI-GST-RPL5	5’ AAGAATTCGGATGGGGTTTGTTAAAGT 3’
3'SalI-GST-RPL5	5’ ATGTCGACTCACCTCCACTTGGC 3’
5'XhoI-GST-RPLP0	5‘ ATCTCGAGATGCCCAGGGAAGAC 3’
3'HindIII-GST-RPLP0	5’ ATAAGCTTTTAGTCAAAGAGACCAAATCC 3’