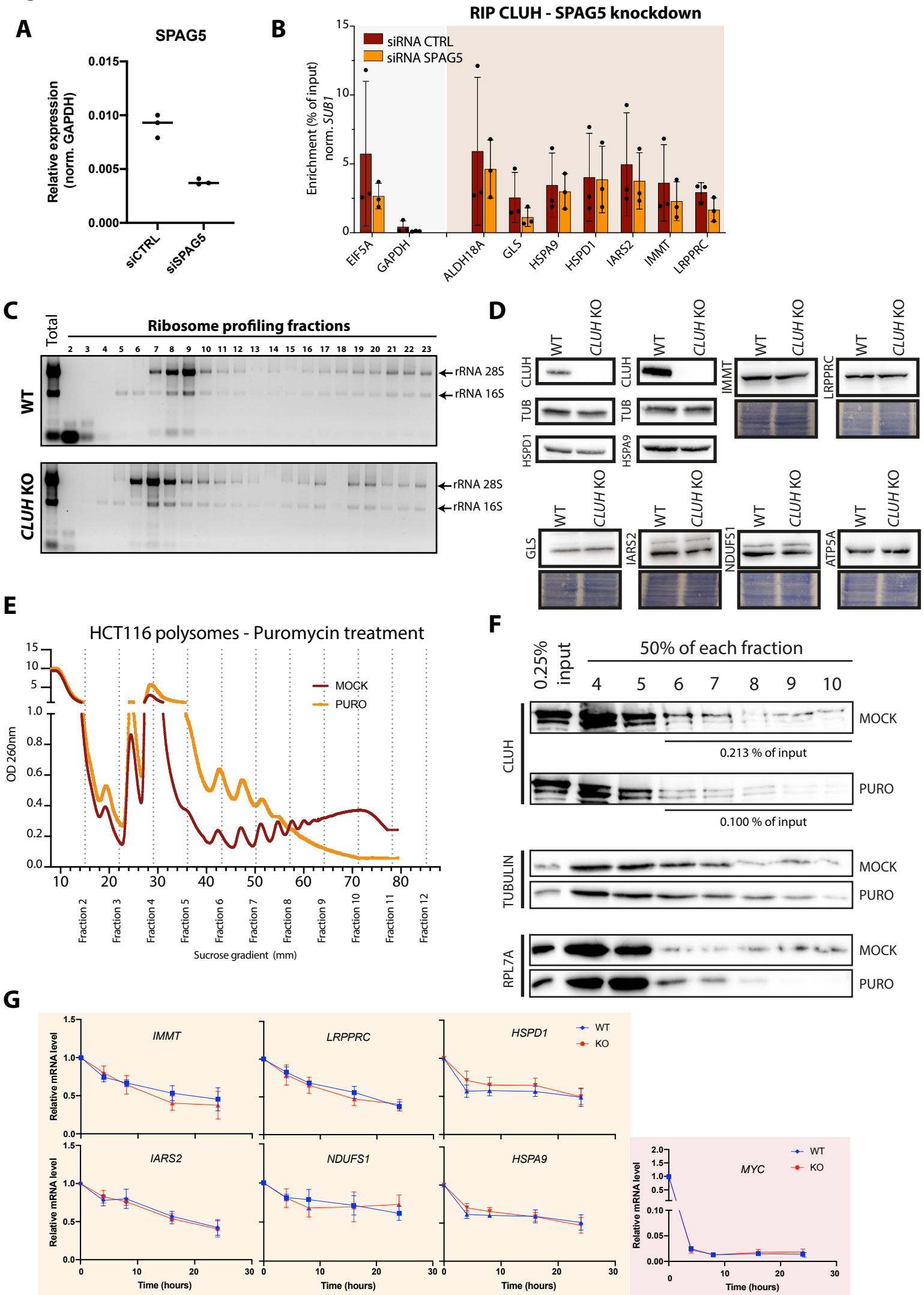


Figure S8



**Figure S8: CLUH effect on RNA stability and translation.**

**(A)** RT-qPCR analysis of SPAG5 expression level in HCT116 cells transfected with either siRNA directed against SPAG5 (siRNA SPAG5) or non-specific siRNA (siRNA CTRL). The level of SPAG5 normalized GAPDH levels in the three independent knock-down experiment, used for the RIP, are shown. Each dot represents the mean value of each experiment. **(B)** RT-qPCR analysis of RIP experiment performed on wild-type HCT116 cells. The CLUH associated mRNAs are enriched using CLUH-specific antibodies and measured by RT-qPCR. The enrichment of specific mRNA (normalized to *SUB1* levels) is calculated relative to the input sample (% of input). mRNAs coding for CPMPs are highlighted by the orange shadow. The error bars correspond to the standard deviation of three independent experiments. The average value for each replicate is indicated by a dot. **(C)** Representative agarose gel electrophoresis analysis of the RNA extracted from the ribosome profiling fractions of both *CLUH* KO and HCT116 cells. The fractions corresponding to about 3.3 mm of the sucrose gradient are numbered from 1 to 23. Input RNA from total extract (Total) is loaded as control. 28S and 16S ribosomal RNA are indicated. RNA is revealed using ethidium bromide staining. **(D)** Western blot analysis of mitochondrial proteins abundance in total protein extracts from *CLUH* KO and WT HCT116 cells. Indicated proteins are revealed using specific antibodies. TUBULIN (TUB) or Coomassie staining of the membrane is used as loading control. **(E)** Representative graphs of polysome profilings of WT HCT116 cells treated (PURO) or not (MOCK) with 100µg/mL of puromycin. The y-axis corresponds to the absorbance at 260 nm and the x-axis to the distance in the sucrose gradient. The collected polysomal fractions are indicated under the x-axis. **(F)** Representative western blot analysis of polysome profiling of WT HCT116 cells treated (PURO) or not (MOCK) with puromycin. About 50% of fractions 4 to 10 are loaded on the gel. Total input protein extracts corresponding to 0.25% of the input extract used to isolate polysomes are used as controls. Indicated proteins are revealed using specific antibodies. The signal of the heaviest polysomal fractions 6 to 10 is indicated relative to the input levels. **(G)** Analysis of mRNA stability over time, after actinomycin D treatment in *CLUH* KO and WT HCT116 cells. The represented mRNA levels are normalized to *GAPDH* levels and are represented relative to time 0. The error bars represent the standard deviation of three replicate experiments. *MYC* is used as a control for the Actinomycin treatment.