Expanded View Figures

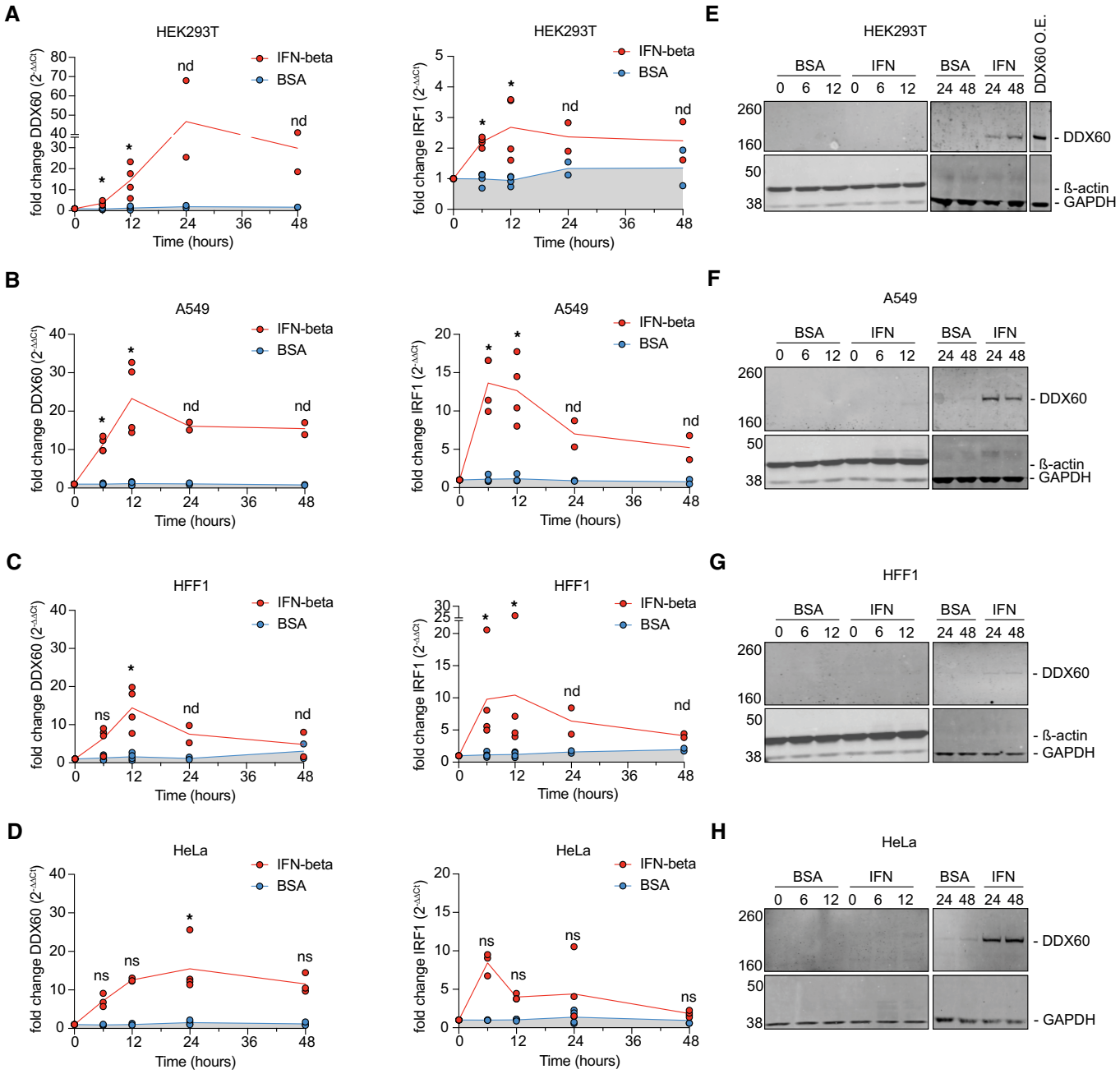

Figure EV1. DDX60 mRNA and protein expression upon interferon treatment.

(A–H) (A, E) HEK293T, (B, F) A549, (C, G) HFF1, or (D, H) HeLa cells treated with either 0.1% BSA (carrier control) or 500 U/ml of interferon-ß for 0, 6, 12, 24, or 48 h. Cells were harvested for mRNA analysis (A–D) using RT-qPCR or protein analysis (E–H) using Western blot. Panel E includes one HEK293T sample transfected with DDX60 wild type run on the same gel as 24- and 48-h time points to show relative DDX60 protein levels in interferon-ß treated versus transfected cells. All western blots for 0-, 6-, and 12-h time points were run on the same gel but are separated by cell line for visualization purposes. All western blots for the 24- and 48-h time points were run on the same gel but are separated by cell line for visualization purposes.

Data information: Individual replicates and mean of mRNA fold-change compared to unstimulated cells. For data points with at least $n = 3$ replicates *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, non-significant (ns) using Mann–Whitney ranked test against carrier control values of same time point. For data points with $n = 2$ replicates statistics were not determined (nd). Representative images of Western blots shown.
Source data are available online for this figure.

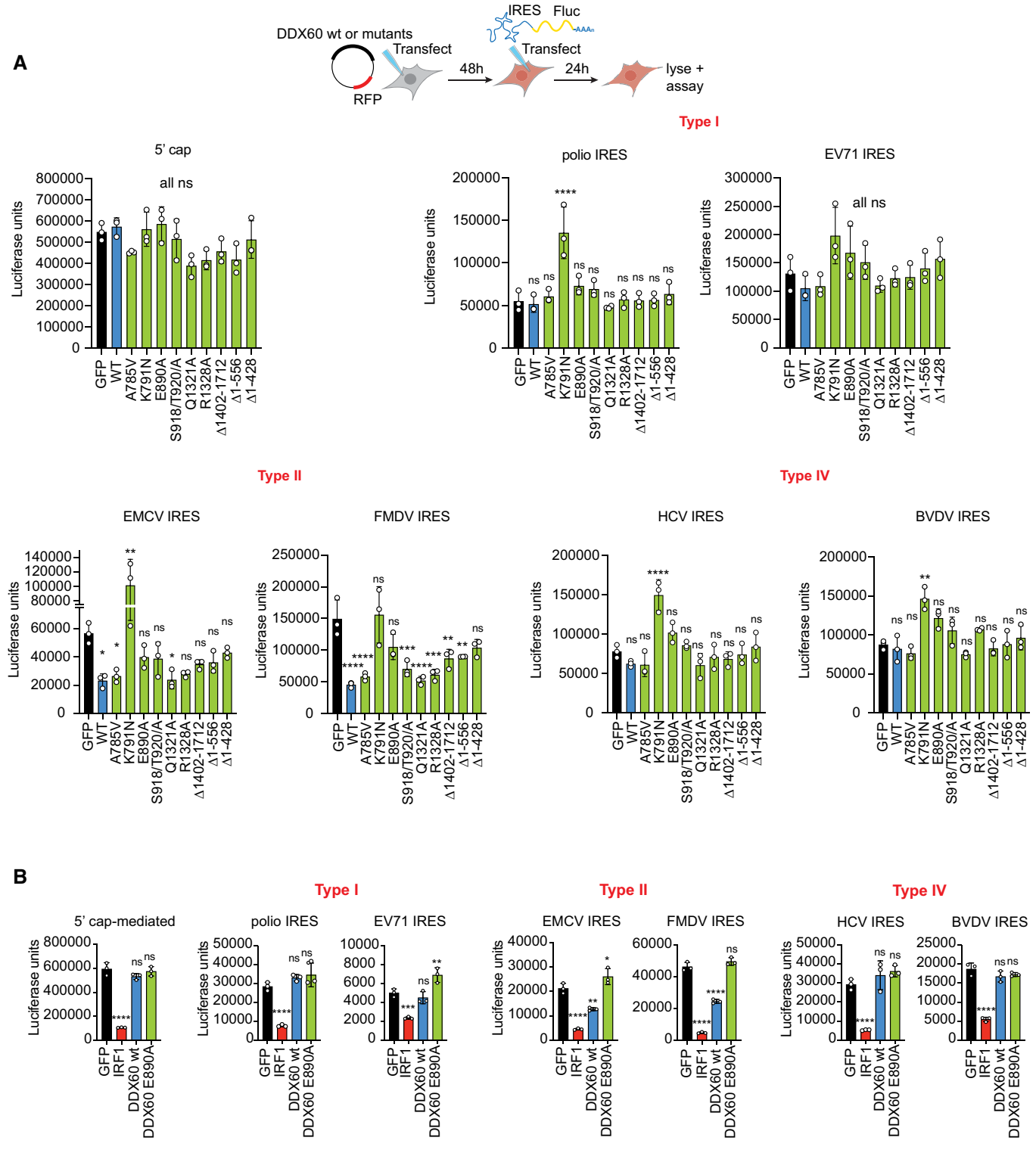

Figure EV2. Monocistronic RNA reporter assays for different internal ribosome entry sites and DDX60 mutant panel.

A HEK293T cells transfected as in Fig 2B and luciferase intensity measured from cell lysates. Mean ± SD for $n = 3$ technical replicates from one representative biological replicate for data shown in Fig 2B; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ using ANOVA and Dunnett's multiple comparison test against GFP.

B HEK293T cells transfected with GFP (negative control), DDX60 wt, or different DDX60 mutants described in Fig 1 and subsequently transfected with *in vitro* transcribed 5′ cap- or different IRES-driven Fluc mRNA constructs and luciferase intensity measured from cell lysates. Mean ± SD of $n = 3$ biological replicates; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ using ANOVA and Dunnett's multiple comparison test against GFP.

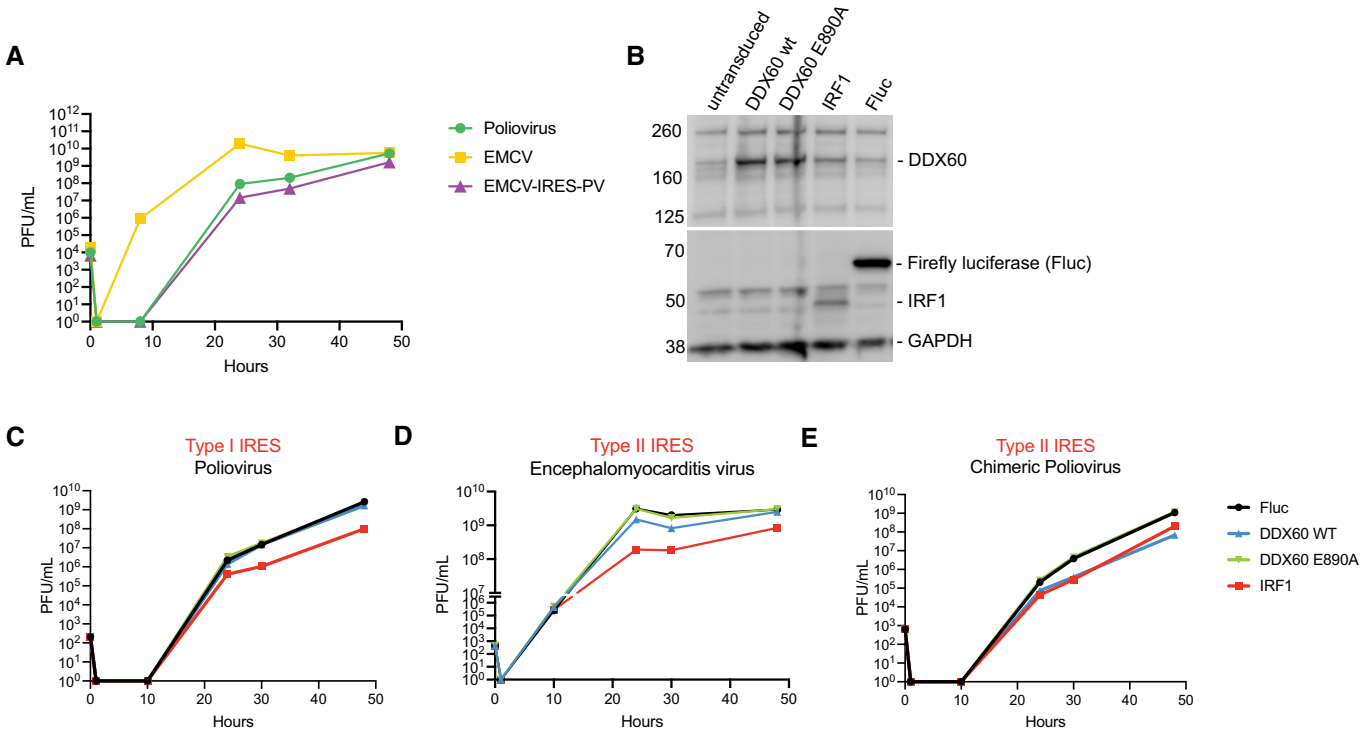

Figure EV3. Growth kinetics of different internal ribosome entry site containing viruses in the presence of DDX60.

A Growth kinetics of poliovirus, encephalomyocarditis virus (EMCV), or a chimeric poliovirus with the poliovirus IRES replaced with the IRES of EMCV. HeLa cells were infected with the three viruses at MOI 0.001 and supernatants from infected cultures were collected 0, 1, 8, 24, 32 or 48 hpi. Virus in supernatants was then titrated on HeLa cells. Mean of $n = 2$ biological replicates.

B Assessment of exogenous DDX60 expression in HeLa cells. HeLa cells were transduced with lentivirus carrying either DDX60 wt, DDX60 E890A, IRF1, or Firefly luciferase (Fluc) together with a puromycin resistance gene and selected with puromycin. Cell lysates were analyzed using western blot for DDX60, Fluc, IRF1, and GAPDH (loading control) protein products.

C–E Time course of antiviral assays with panel of IRES-containing viruses. HeLa cells stably expressing Fluc (negative control), IRF1 (positive control), DDX60 wt, or DDX60 E890A were infected with (C) poliovirus, (D) EMCV, or (E) a chimeric poliovirus at a MOI of 0.001. Supernatants from infected cultures were collected at either 0, 1, 8, 24, 32, or 48 hpi and titrated on HeLa cells via plaque assay. Mean of $n = 2$ biological replicates.

Source data are available online for this figure.

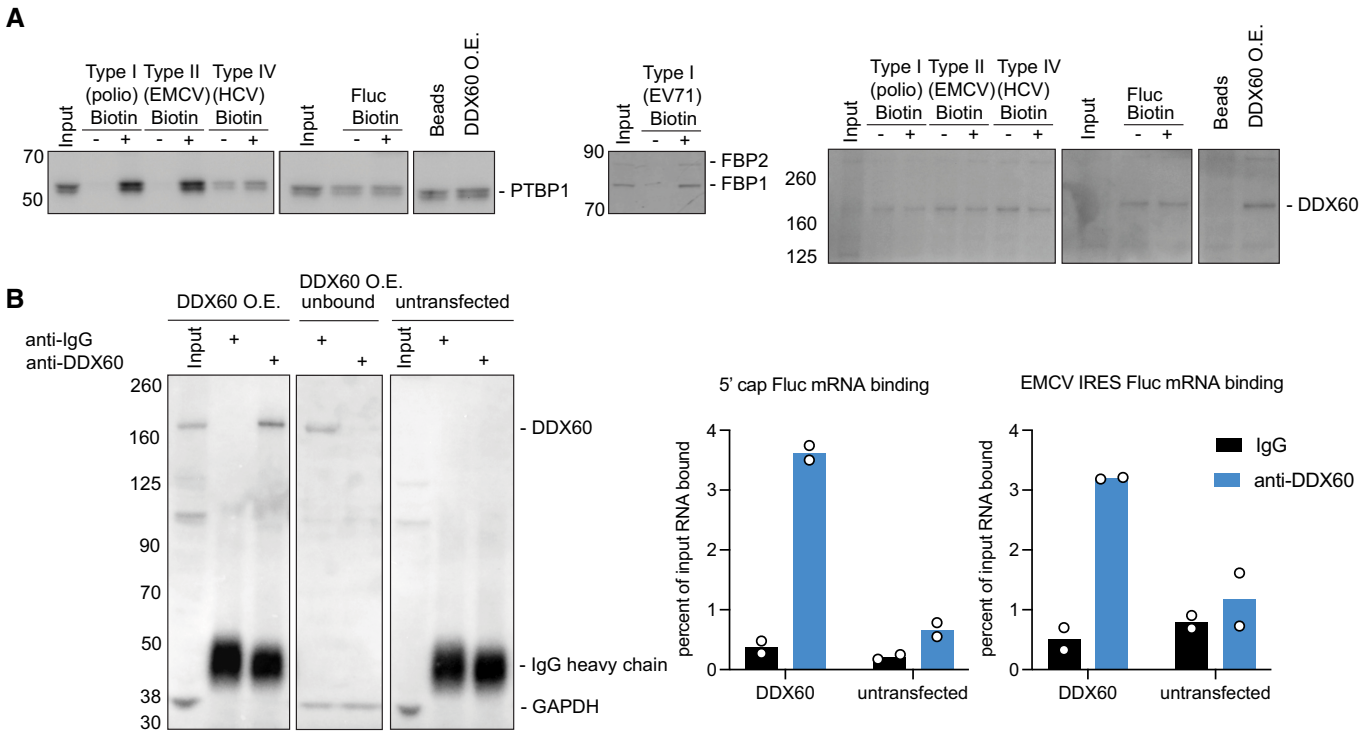

Figure EV4. Probing for a physical interaction between DDX60 and different IRES sequences.

A RNA-protein binding assays using biotin labeled RNA probes and streptavidin coated beads. DDX60 wt expressing HEK293T cell lysates were combined with either biotin labeled or unlabeled (negative control) IRES RNA sequences (IRESs from poliovirus, EV71, EMCV, or HCV) or 5′ capped Fluc RNA sequence (negative control). After allowing for protein and RNA binding, streptavidin-coated beads were used to pull down RNA. After washing, RNA bound proteins were visualized using Western blot. Input or DDX60 overexpression (O.E) lanes show DDX60 and other interrogated RNA binding proteins before incubation of cell lysates with interrogated RNAs. Comparisons for enrichment of RNA binding is made by comparing unlabeled and biotin labeled lanes. Visually equivalent band intensities between unlabeled and biotin labeled lanes signify nonspecific binding between an RNA and interrogated protein. Lane labeled beads represents amount of protein that binds to streptavidin coated beads in the absence of any interrogated RNA. RNA binding proteins interrogated include Polypyrimidine Tract Binding Protein 1 (PTBP1) (positive control for binding type I and type II IRESs specifically), Far upstream element binding protein 1 (FBP1) (binds EV71 IRES), and DDX60. Data is representative of $n = 2$ biological replicates.

B Protein-RNA binding assays using immunoprecipitated DDX60 and RT-qPCR for Fluc mRNAs. HEK293T cells transfected with DDX60 wt or left untransfected were transfected with either 5′ cap- or EMCV IRES-driven Fluc mRNA. Cell lysates were then subjected to immunoprecipitation using either DDX60 targeting antibody or isotype control IgG. Bound mRNA was detected using RT–qPCR against Fluc. Efficiency of immunoprecipitation is visualized using western blot against DDX60. Bound mRNA is quantified as a percent of input RNA postimmunoprecipitation with either IgG or anti-DDX60 antibody in either DDX60 expressing or untransfected cells. Shown are a representative blot and mean of $n = 2$ biological replicates.

Source data are available online for this figure.

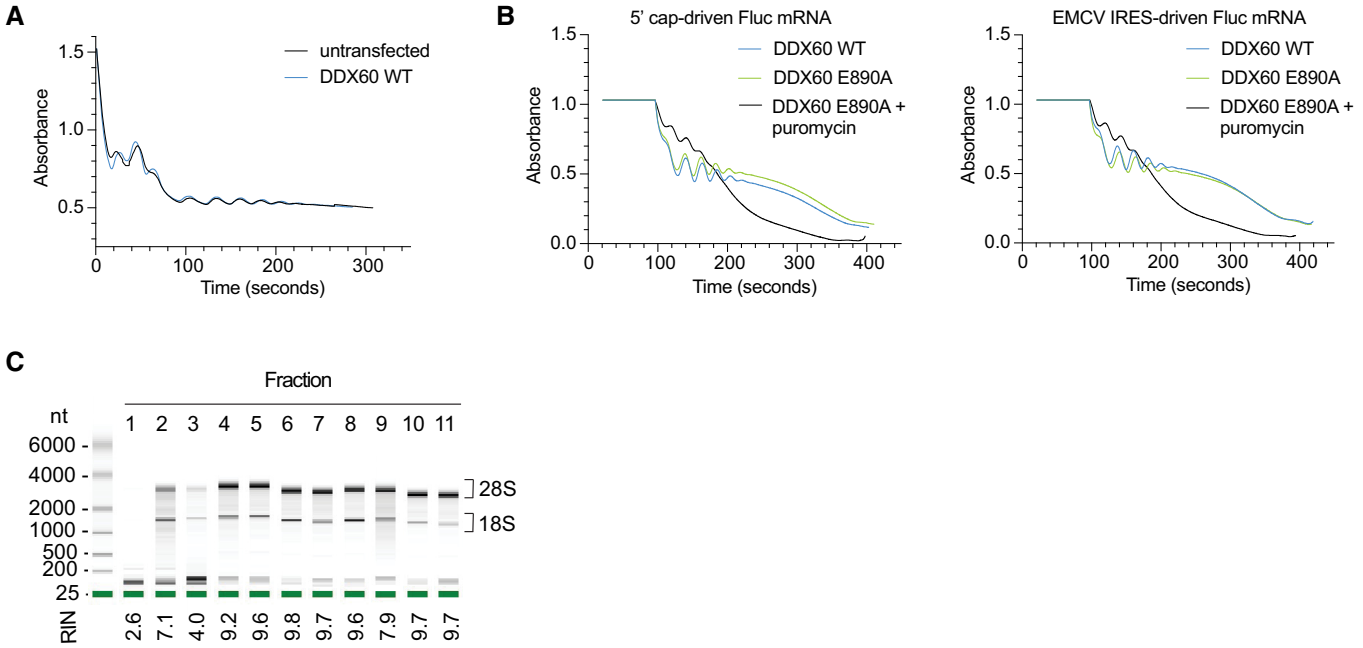

Figure EV5. Global translation levels under DDX60 overexpression.

A Polysome profile of total RNA upon DDX60 overexpression. Untransfected or DDX60 wt expressing HEK293T cells were treated with 100 μg/ml of cycloheximide for 15 min to arrest polysomes before being trypsinized and frozen down. All harvested cells per condition were then subjected to polysome profiling by lysing cells in the presence of cycloheximide and protease and phosphatase inhibitors, applying cell lysates to 15–50% sucrose gradients, and subjecting to ultracentrifugation. The centrifuged gradients were run through a fractionator and total RNA in each fraction was measured by UV absorbance (254 nm). Data are an average of two replicate plate of cells per condition.

B Polysome profile of total RNA post transfection of DDX60 and Fluc mRNA reporters. DDX60 wt or DDX60 E890A (negative control) expressing HEK293T cells transfected with *in vitro* transcribed 5′ cap (left) or EMCV IRES (right) driven Fluc mRNA constructs. Duplicate samples of DDX60 E890A and 5′ cap- or EMCV IRES-driven Fluc mRNA transfected cells were treated with 200 μM puromycin as positive controls for decrease in polysomes. Cells were then treated with 100 μg/ml of cycloheximide for 15 min to arrest polysomes before being trypsinized and frozen down. All harvested cells per condition were then subjected to polysome profiling by lysing cells in the presence of cycloheximide and protease and phosphatase inhibitors, applying cell lysates to 15–50% sucrose gradients, and subjecting to ultracentrifugation. The centrifuged gradients were run through a fractionator and total RNA in each fraction was measured by UV absorbance (254 nm). Mean of $n = 3$ biological replicates.

C Representative results of electrophoresis analysis of individual fractions. Shown is RNA from one replicate of DDX60 E890A and cap-fluc transfected cells (control). RNA was analyzed by Bioanalyzer to visualize 28S and 18S ribosome subunit distribution in the different fractions. RIN, RNA integrity number.