Expanded View Figures

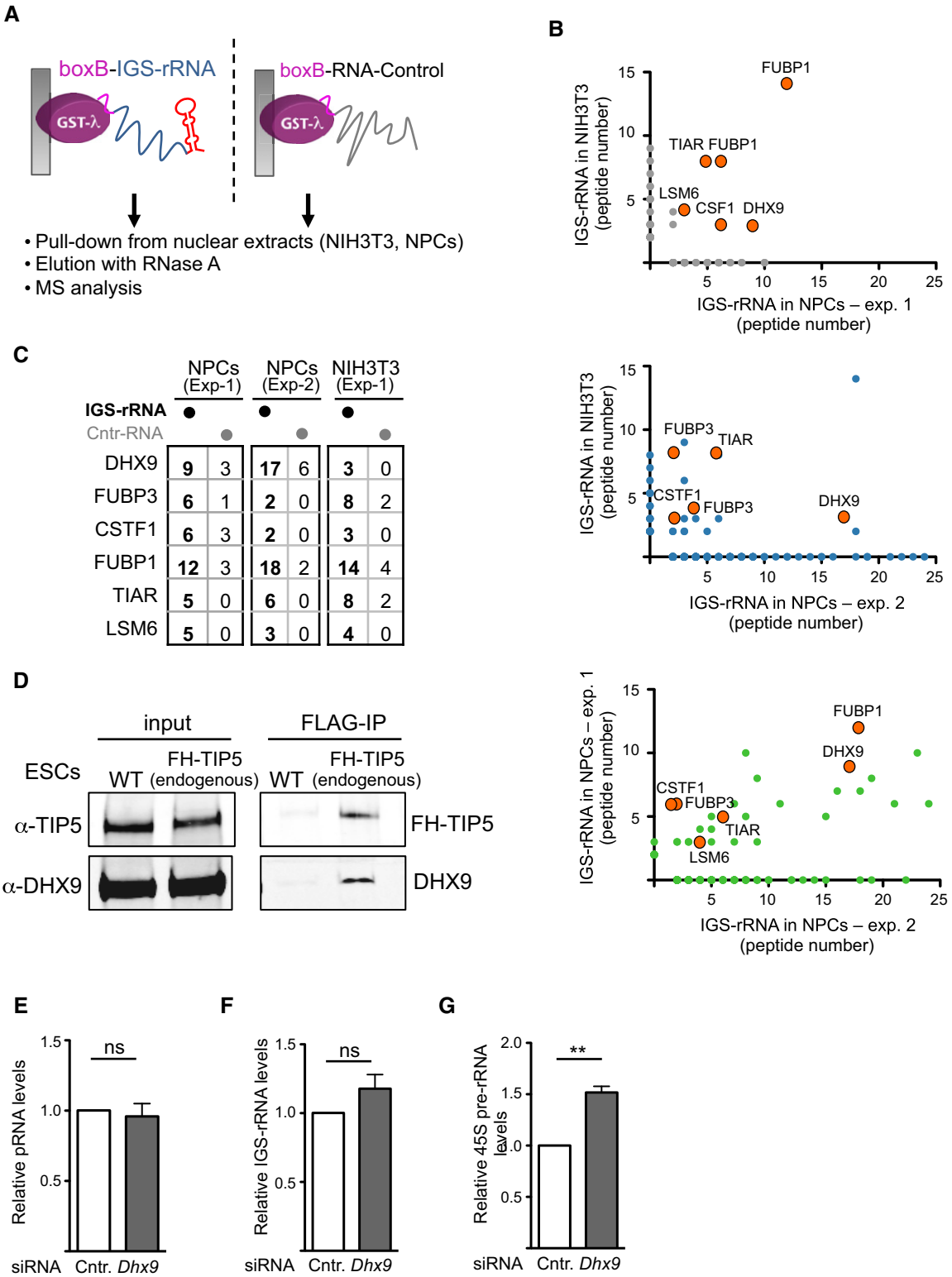

Figure EV1.

Figure EV1. DHX9 associates with IGS-rRNA and TIP5.

A Schema represents the GRNA chromatography employed for the identification of IGS-rRNA-binding proteins.

B Mass spectrometry analysis of proteins pulled down with IGS-rRNA and found enriched in all three independent experiments, which used nuclear extracts from NIH 3T3 cells or NPCs. Values refer to peptide numbers obtained in each experiment (gray, blue and green circles). Orange circles highlight the six IGS-rRNA-binding proteins found to associate with IGS-rRNA in all experiments.

C Comparison of peptides pulled down with IGS-rRNA in three independent experiments using nuclear extracts from NIH 3T3 cells and NPCs. Values refer to peptide numbers obtained in each experiment.

D Co-IP showing the interaction of endogenous TIP5 and DHX9 in embryonic stem cells (ESCs). Flag-HA (FH)-TIP5 ESCs were obtained by co-transfection of the CRISPR/Cas9 vector expressing an sgRNA targeting the TIP5 genomic locus 3 nt upstream of the ATG start codon with a homology directed repair (HDR) template. Immunoblots of Flag-IP and input show the association of endogenous FH-TIP5 with DHX9.

E–G Knockdown of DHX9 upregulates 45S pre-rRNA levels but does not affect the total amount of IGS-rRNA and pRNA. qRT–PCR from NIH 3T3 cells transfected with siRNA-control and siRNA-DHX9. Values were obtained by amplification of cDNA generated in reverse transcription reactions using random hexamers. Values (mean ± SD) were from four independent experiments and calculated relative to *GAPDH* mRNA amounts and to control cells (siRNA-control). Statistical significance (*P*-values) for the experiments was calculated using the paired two-tailed *t*-test (**$P < 0.01$; ns, non-significant).

Source data are available online for this figure.

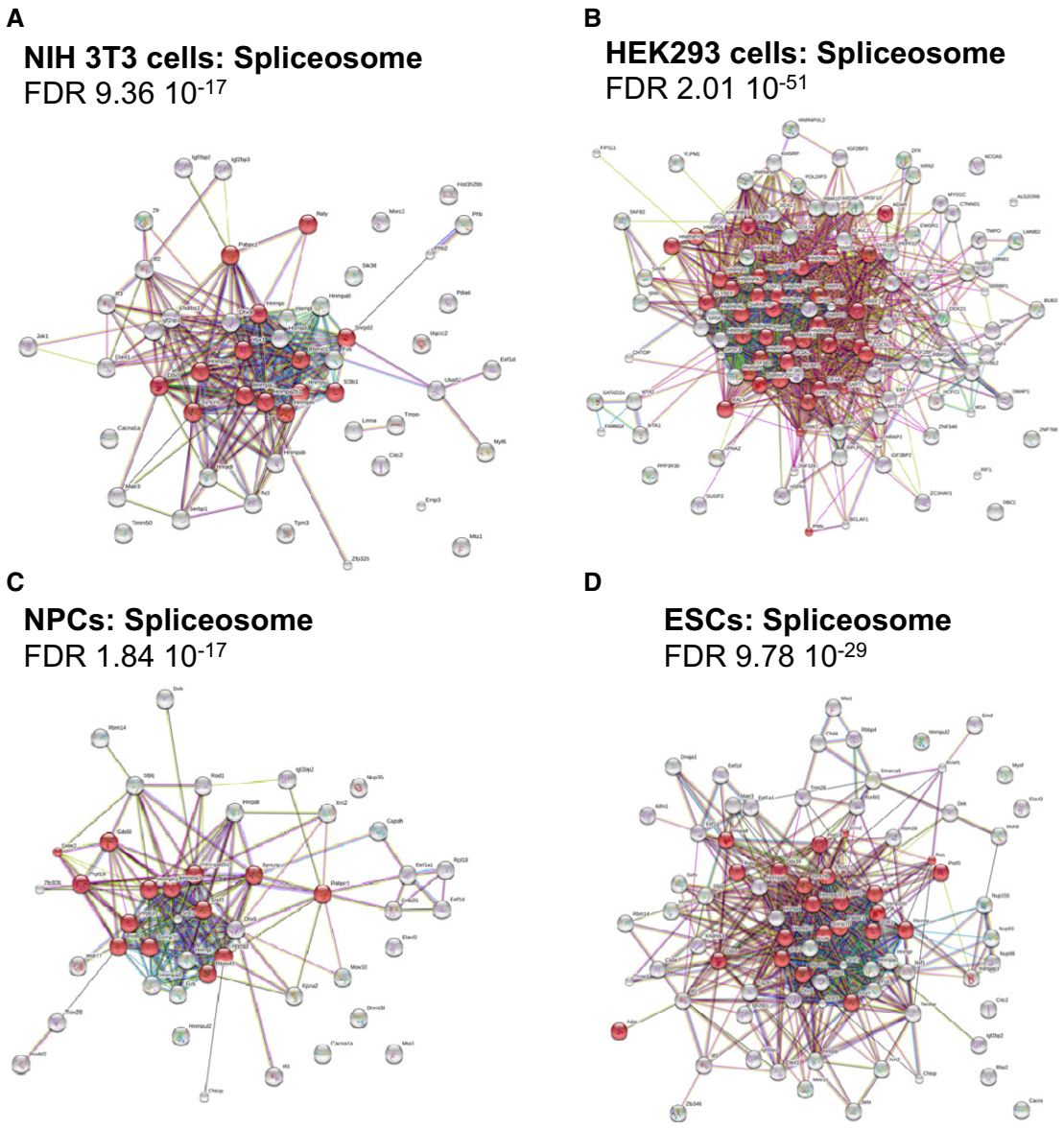

Figure EV2.

Figure EV2. DHX9 associates with components of RNA processing and spliceosome.

A–D STRING analysis depicting functional protein association networks of DHX9 interacting proteins found in NIH 3T3 cells (A), HEK293T (B), ESCs after 3-day differentiation (C), and ESCs (D). DHX9-interacting proteins were identified by transfection of FLAG-DHX9 plasmid, followed by anti-FLAG immuno-precipitation, FLAG peptide elution, and mass spectrometry analysis. Identified proteins are listed in Dataset EV1. Biological pathways and KEGG analysis are in Dataset EV2.

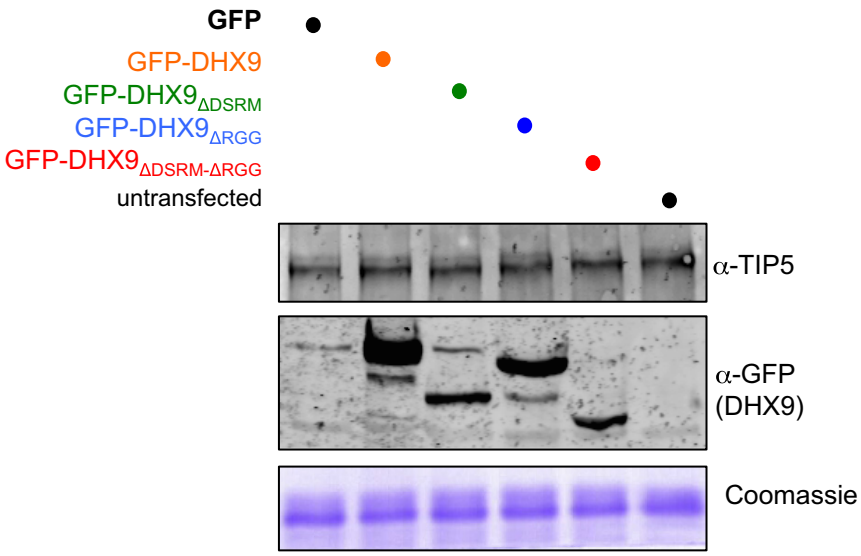

Figure EV3. Ectopic expression of DHX9 wild type and mutants does not affect TIP5 endogenous levels.

Western blot of lysates of HEK293 cells transfected with the indicated DHX9 expression vectors. Coomassie staining serves as control for equal loading between samples.

Source data are available online for this figure.

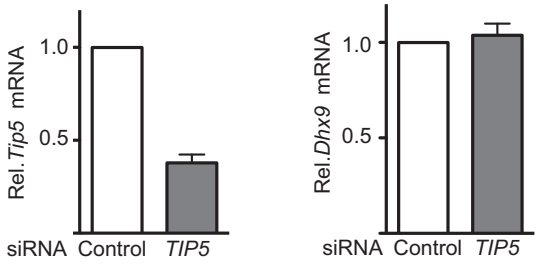

Figure EV4. TIP5 knockdown does not affect DHX9 levels.

mRNA of TIP5 and DHX9 in NIH 3T3 cells depleted of TIP5 by siRNA. TIP5 mRNA values were normalized to *GAPDH* mRNA and to siRNA-control cells. Average and standard deviation ($n = 3$) are shown for each point.

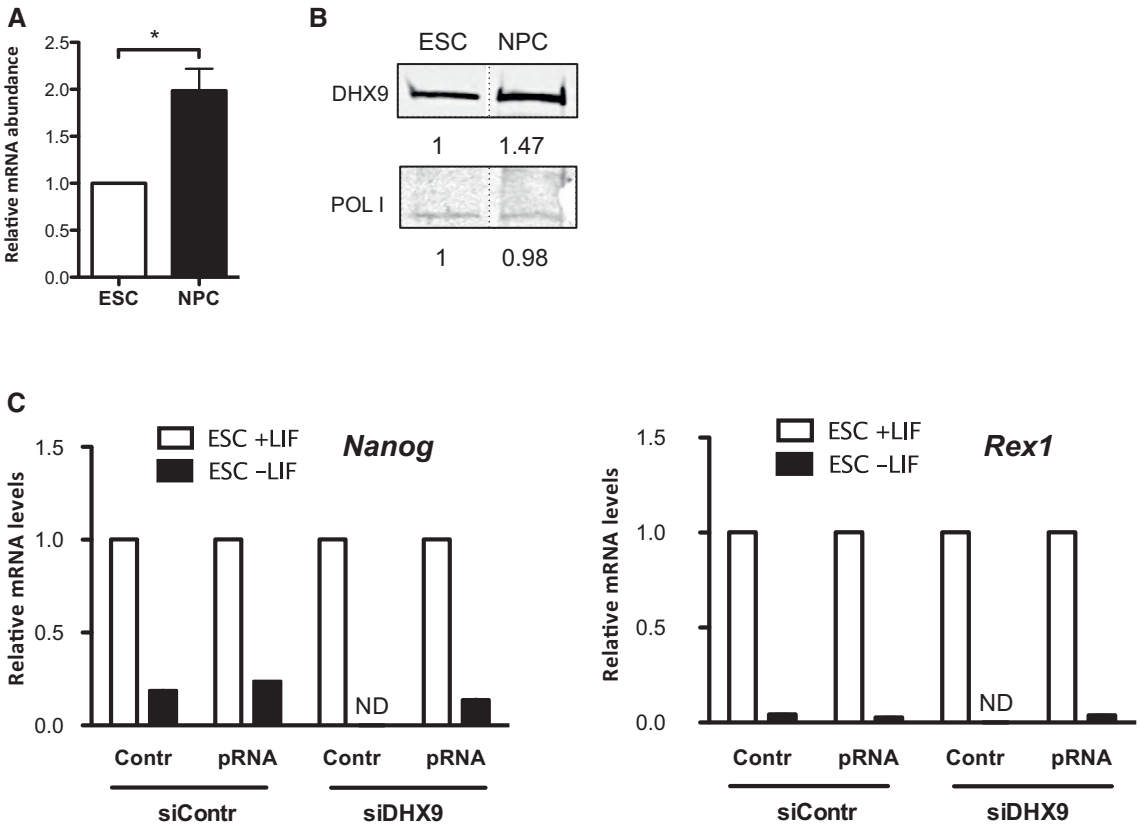

Figure EV5. Expression levels of DHX9 in ESCs and NPCs.

A, B DHX9 transcript (A) and protein (B) levels in ESCs and NPCs. RT–qPCR values (mean ± SD) from three independent experiments were normalized to *Rps12* mRNA levels and to ESCs. Statistical significance (*P*-values) for the experiments was calculated using the paired two-tailed *t*-test (*$P < 0.05$).

C qRT–PCR showing expression levels of pluripotency markers *Nanog* and *Rex1* in ESCs + LIF and differentiated cells obtained upon withdrawal of LIF (ESCs−LIF). Values were normalized to *rsp12* mRNA and the corresponding ESCs. Because of the few amounts of differentiated cells obtained upon DHX9 knockdown, mRNA levels in these samples could not be determined (ND). Data represent the mean of two independent experiments.

Source data are available online for this figure.