Expanded View Figures

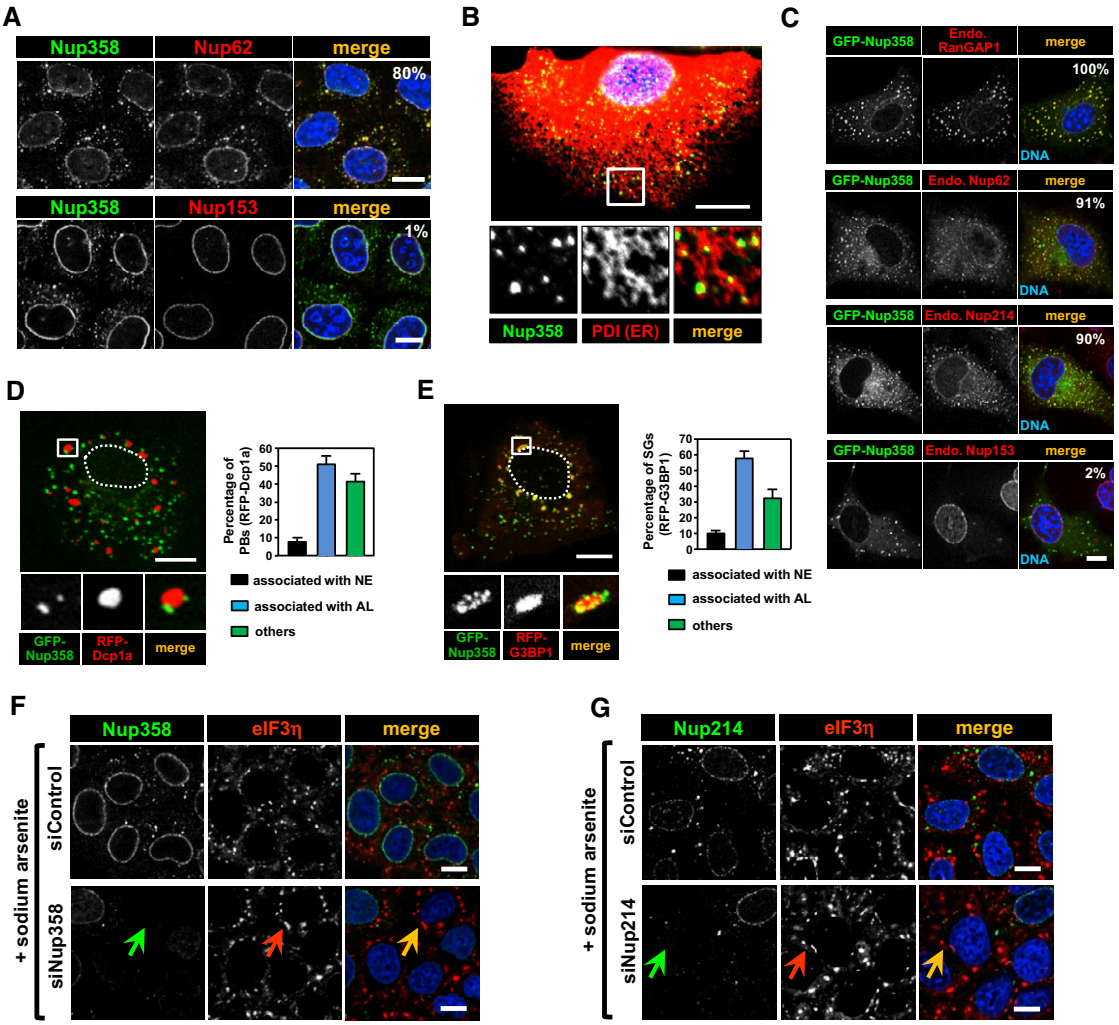

Figure EV1. Endogenous and overexpressed Nup358-positive cytoplasmic puncta represent AL.

A HeLa cells were immunostained for Nup62 (red, upper panel) or Nup153 (red, lower panel) along with Nup358 (green) using specific antibodies. DNA was stained with Hoechst 33342 (blue, merge). Percentages of Nup358-positive cytoplasmic puncta co-localizing with Nup62 (upper panel) and Nup358 with Nup153 (lower panel) are indicated. Scale bars, 10 μm.

B HeLa cells were fixed and stained for endogenous Nup358 (green) and PDI (red, as an ER marker). Scale bar, 10 μm.

C COS-7 cells were transfected with GFP-Nup358 (green) and stained for endogenous RanGAP1, Nup214, Nup62, or Nup153 (red) using specific antibodies. DNA is shown in blue (merge). Percentages of GFP-Nup358-positive cytoplasmic puncta co-localizing with respective proteins are indicated. Scale bar, 10 μm.

D COS-7 cells expressing GFP-Nup358 (green) and RFP-Dcp1 (red) were fixed and analyzed for their relative distribution within the cytoplasm. Often GFP-Nup358-positive AL structures are in close proximity/associated with P bodies (RFP-Dcp1a). The region marked by square is magnified (inset). Dotted line indicates nuclear periphery. Graph represents quantitative data showing percent of P bodies (RFP-Dcp1a) associated with nuclear envelope (NE), GFP-Nup358-positive AL, or unassociated with either of the structures (free). Scale bar, 10 μm. Data are presented as mean ± SD (n = 3).

E COS-7 cells expressing GFP-Nup358 (green) and RFP-G3BP1 (red) were fixed and analyzed by fluorescence microscopy. Often GFP-Nup358-positive AL structures are in close proximity/associated with SGs induced by RFP-G3BP1 expression. The region marked by square is magnified (inset). Dotted line indicates nuclear periphery. Graph represents quantitative data showing percent of SGs (RFP-G3BP1) associated with nuclear envelope (NE), GFP-Nup358-positive AL, or unassociated with either of the structures (free). Scale bar, 10 μm. Data are presented as mean ± SD (n = 3).

F HeLa cells were transfected with control siRNA (siControl) or siRNA against Nup358 (siNup358). Cells were treated with 0.5 mM sodium arsenite for 30 min prior to fixation and stained for endogenous Nup358 (green) and eIF3η (red) as a SG marker using specific antibodies. DNA was stained with Hoechst 33342 (merge, blue). Arrows indicate a Nup358-depleted cell. Scale bars, 10 μm.

G HeLa cells were transfected with control siRNA (siControl) or Nup214 siRNA (siNup214). Cells were treated as indicated in (A) and were fixed and stained for endogenous Nup358 (green) and a SG marker (eIF3η, red). DNA was stained with Hoechst 33342 (merge, blue). Arrows indicate a Nup214-depleted cell. Scale bars, 10 μm.

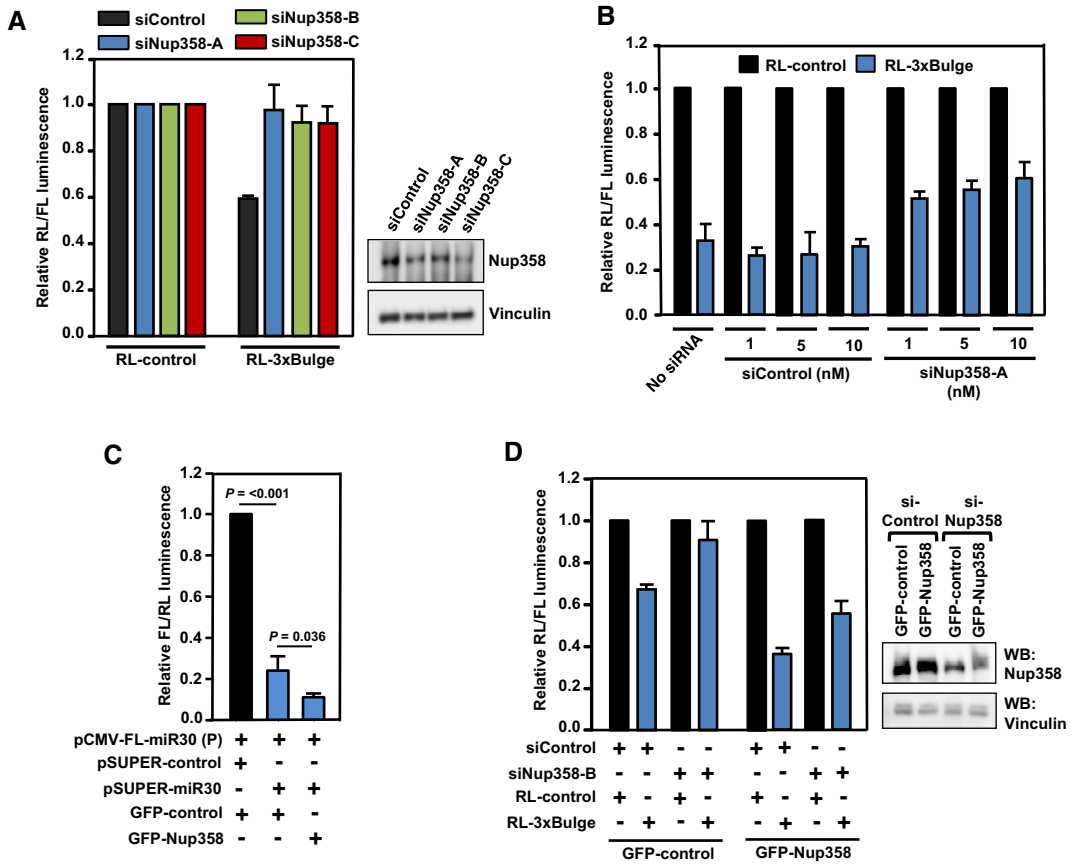

Figure EV2. Nup358 depletion impairs and overexpression enhances miRNA-mediated mRNA suppression.

A HeLa cells were transfected with siRNAs targeted against different regions of Nup358 transcript (siNup358-A, siNup358-B, or siNup358-C), and subsequently with indicated RL reporter constructs along with FL as internal control. Left panel: RL/FL luminescence ratio was calculated. Data are presented as mean ±SD (n = 3). Right panel: Western analysis of lysates prepared from cells transfected with three different siRNAs as indicated, for monitoring the extent of Nup358 depletion. Vinculin was used as loading control. Nup358-A was used for depleting Nup358 (as siNup358) in all the experiments in the study, except where mentioned otherwise.

B HeLa cells were initially transfected with no (no siRNA), or varying concentrations of control (siControl) or Nup358-specific (siNup358) siRNA in different concentrations (in nM) as indicated. Subsequently, the cells were transfected with RL-3xBulge reporter along with FL as internal control. RL/FL luminescence ratio was calculated. Data are presented as mean ± SD (n = 3).

C HeLa cells were co-transfected with indicated constructs and dual-luciferase assay was performed. RL/FL luminescence ratio was calculated. Data are presented as mean ± SD (n = 3), P-values were calculated using Student's t-test.

D HeLa cells were sequentially transfected with control (siControl) or siRNA targeting the 3′-UTR of Nup358 (siNup358-B), followed by RL-3xBulge reporter construct, along with FL as internal control. These were co-transfected with either GFP-control or GFP-Nup358 constructs. Left panel: RL/FL luminescence ratio was calculated. Data are presented as mean ± SD (n = 3). Right panel: Western blot analysis of the lysates prepared from cells transfected with indicated siRNAs and expression constructs, using anti-Nup358 antibodies. Vinculin was used as loading control.

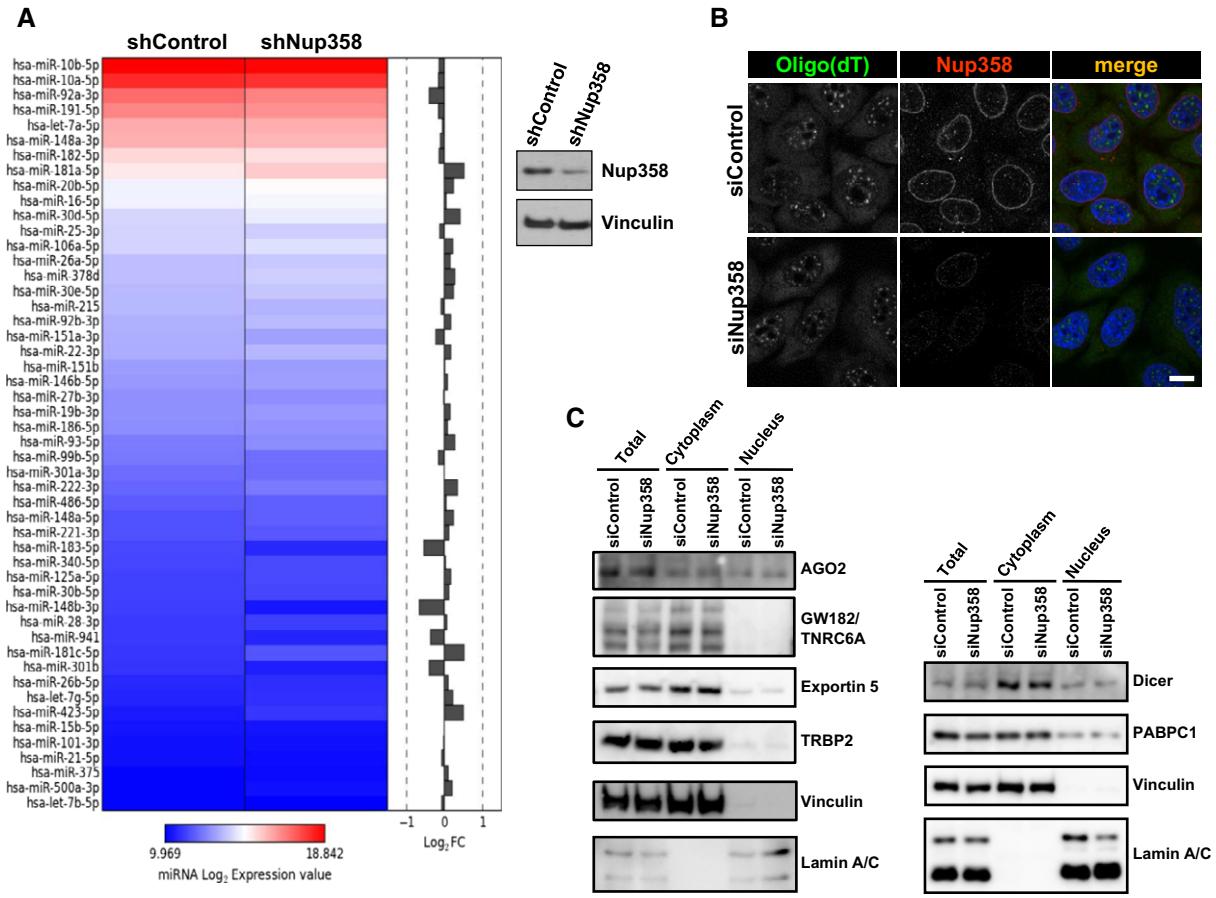

Figure EV3. Nup358 depletion does not grossly alter mature miRNA levels or nucleo-cytoplasmic distribution of mRNAs and key proteins involved in miRNA pathway.

A HEK293T cells were transfected with shRNA control (shControl) or shRNA against Nup358 (shNup358). Heatmap shows relative levels of 50 most abundant miRNAs detected between shControl and shNup358-transfected HEK293T cells. Western blot (right panel) shows the extent of Nup358 depletion. Vinculin was used as loading control.

B HeLa cells were transfected with control (siControl) or Nup358 (siNup358)-specific siRNA. Cells were fixed and stained for mRNA using labeled oligo(dT) (red) and for Nup358 (green) using specific antibodies. DNA was stained with Hoechst 33342 (blue, merge). Scale bar, 10 μm.

C HeLa cells were subjected to control (siControl) or Nup358 (siNup358) depletion and the nucleo-cytoplasmic fractionation was performed and analyzed for different proteins as indicated. Vinculin and lamin A/C were used as cytoplasmic and nuclear markers, respectively. Right and left panels represent two independent fractionations.

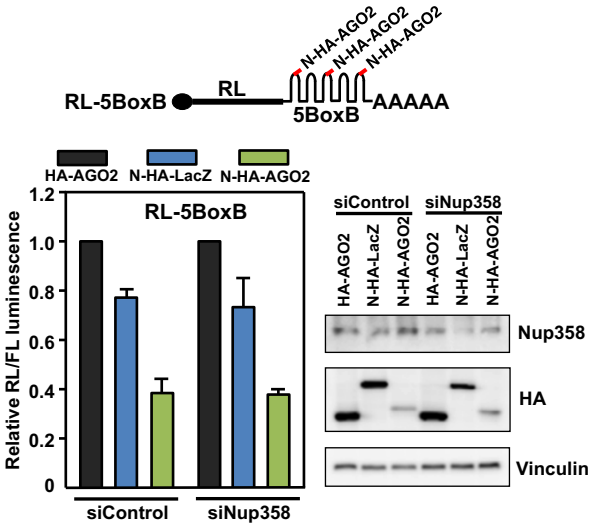

Figure EV4. Nup358 depletion does not affect repression of mRNAs tethered with AGO2.

HEK293T cells were sequentially transfected with control (siControl) or Nup358 siRNA (siNup358), followed by co-transfection of pRL-5BoxB construct (a representative schematic is shown on top) with FL as internal control, and HA-AGO2, N-HA-LacZ, or N-HA-AGO2, as indicated. Left panel: Graph represents RL/FL luminescence normalized to HA-AGO2 transfected samples for control and Nup358 knockdown, separately. RL/FL luminescence ratio was calculated. Data are presented as mean ± SD (n = 3). Western blot (right panel) shows the extent of Nup358 knockdown in cells transfected with indicated siRNA and expression constructs, using specific antibodies. Vinculin was used as loading control.

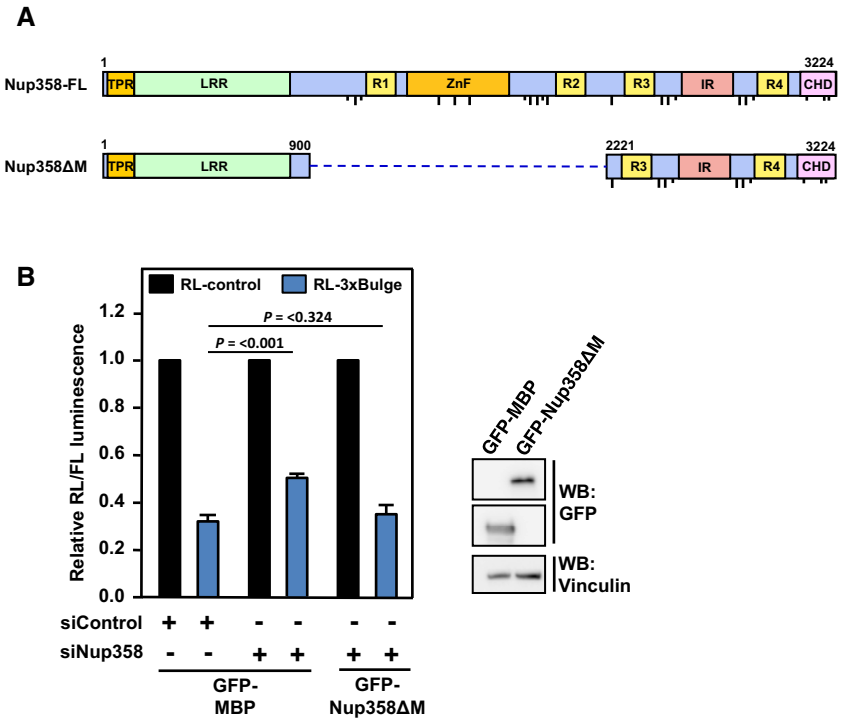

Figure EV5. The middle region of Nup358, consisting of the RNA-binding RanBP2-type zinc fingers, is dispensable for miRNA function.

A The domain architecture of human full-length (FL) Nup358. TPR, tetratricopeptide repeat; LRR, leucine-rich region; R1–R4, RanGTP-binding domain; ZnF, zinc finger domains; IR, internal repeats; CHD, cyclophilin-homology domain. Dotted line indicates the deleted region in Nup358ΔM.

B HeLa cells were transfected with control (siControl) or Nup358 (siNup358)-specific siRNAs. Subsequently, RL-3xBulge or RL-3xBulge-mut reporter construct was transfected along with GFP-maltose binding protein (MBP, control) or GFP-Nup358ΔM construct. The dual-luciferase assay was performed to monitor the miRNA activity. FL was used as a transfection control. Left panel: RL/FL luminescence ratio was calculated. Data are presented as mean ± SD (n = 3), P-values were calculated using Student's t-test. Right panel: Western blot showing relative expression of indicated constructs in HEK293T cells using anti-GFP antibodies.