Appendix-Supplementary Information

Elucidation of Nup358's role in miRNA pathway reveals a new motif for interaction with AGO family of proteins

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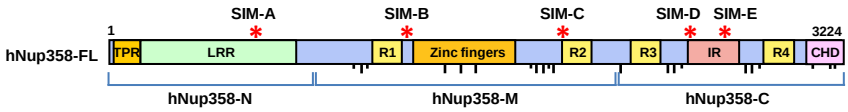
List of Content

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SIM-A:        N L A L I F H R K A (682-691 aa)
SIM-B:        A V R I V K E P T S (1319-1328)
SIM-C:        K V E L V T G E E D (2022-2031)
SIM-D(SIM1): D V L I V Y E L T P (2631-2640 aa)
SIM-E(SIM2): E C I I V W E K K P (2710-2719 aa)
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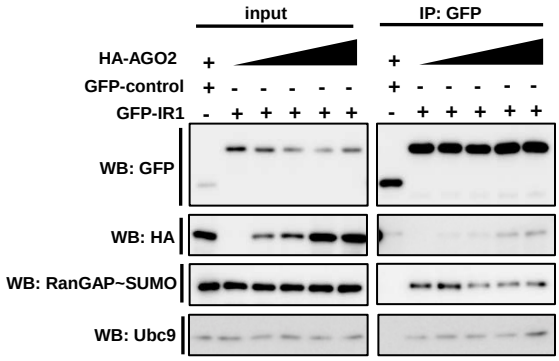
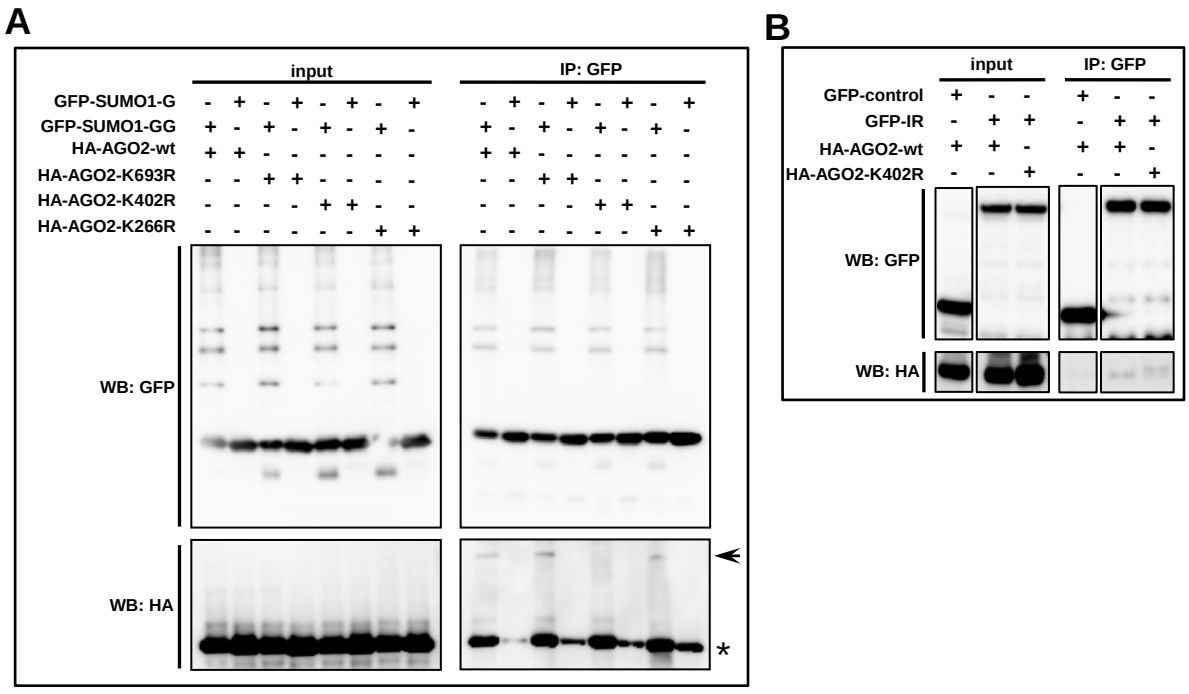
Appendix Figure S1. Presence of multiple SIMs in Nup358

Human Nup358 has five SIMs as predicted by GPS-SUMO program using medium threshold values. Top part is the schematic diagram of Nup358 domains, with corresponding regions in N, M and C fragments marked. * indicates the position of individual potential SIM. Lower part is the aligned SIMs, with conserved hydrophobic residues implicated in SUMO binding shown in red. SIM-D and SIM-E are validated SIMs and they correspond to SIM1 and SIM2 used in this study.



Appendix Figure S2. AGO2 competes with SUMO~RanGAP for interaction with IR1

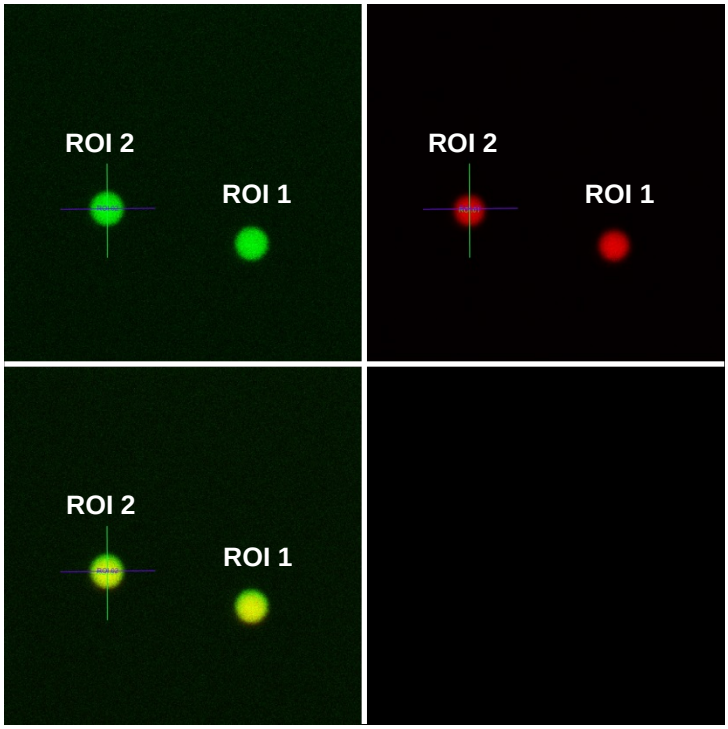
HEK293T cells were co-transfected with GFP-control or GFP-IR1 and increasing amounts of HA-AGO2 constructs as indicated. The cell lysates were subjected to co-immunoprecipitation using GFP-specific antibodies and the immunoprecipitates were probed for the presence of ectopically expressed AGO2 using HA-specific antibodies. Endogenous SUMO~RanGAP and Ubc9 in the input and immunoprecipitates were detected with specific antibodies.

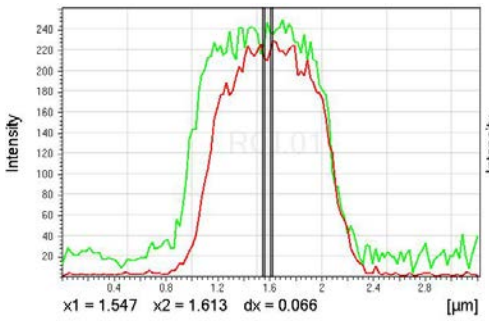


Appedix Figure S3. AGO2 interacts with Nup358-IR in a SUMOylation independent manner

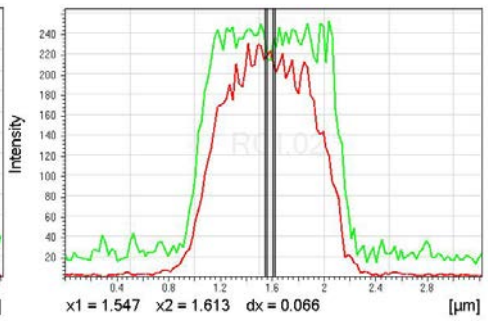
A. HEK293T cells were transfected with GFP-SUMO1-G or GFP-SUMO1-GG and indicated HA-tagged AGO constructs. The cell lysates were subjected to immunoprecipitation using GFP-specific antibodies and the immunoprecipitates were probed for the presence of ectopically expressed AGO2 using HA-specific antibodies. Arrow indicates SUMO-modified AGO proteins and asterisk represents unmodified AGO proteins.

B. HEK293T cells expressing GFP or GFP-IR and HA-AGO2-wild type (wt) or HA-AGO2-K402R mutant were lysed and immunoprecipitation assay was performed using GFP antibodies. Presence of HA-AGO2 in the immunoprecipitate was assessed by western blot analysis using HA antibodies.





Region of Interest 1 (ROI 1)



Region of Interest 2 (ROI 2)

Appendix Figure S4. Testing the Images obtained from Leica SP5 confocal microscope for alignment / chromatic aberration between two channels.

Images of TetraSpeck beads (1 μm size) labelled with two fluorophores having excitation with 405 nm laser and 561 nm laser and emission 420 to 480 nm (green) and 570 to 630 nm (red), respectively, were obtained. Images were captured in line sequential mode. Similar results were obtained in simultaneous mode as well. Graphs show intensity profiles for the co-localization of two colors for two separate beads (ROI 1 and ROI 2).