Supplementary Figure S1.

Overexpression of untagged Ago2 inhibits the nuclear transport of the dotted foci of GFP-signal of myc-GFP-TNRC6A-NES-mut. (A–C) HeLa cells expressing myc-GFP-TNRC6A-NES-mut with either of FLAG/HA-FL (A), -Ago2 (B), or untagged Ago2 (C) were stained with an anti-Ago2 antibody, followed by Cy5-conjugated anti-mouse IgG. Fluorescent images are shown from the left: GFP signals of myc-GFP-TNRC6A-NES-mut; Cy5 signals of the second antibody against anti-Ago2 antibody; the merged images of GFP and Cy5, in which GFP is shown in green, the Cy5 in magenta; the merged images with DAPI (blue). Note that anti-Ago2 positive signals indicate endogenous Ago2 (A), endogenous and FLAG/HA-tagged Ago2 (B), and endogenous and untagged Ago2 (C). Bars, 20 μm. (D) The ratio of cells expressing the dotted GFP-signal of myc-GFP-TNRC6A-NES-mut exclusively in the nucleus (N, blue), cytoplasm (C, red), or both (N+C, yellow). (E) Cell lysates were analyzed by western blot using an anti-Ago2 antibody, an anti-FLAG antibody and an anti-GFP antibody. An anti-tubulin was used as a loading control.
Supplementary Figure S2.

Overexpression of Ago2 inhibits the nuclear transport of the dotted GFP-signal of myc-GFP-TNRC6A by the treatment of LMB via Ago-binding GW motifs in TNRC6A. (A) Schematic representation of the domain structure of wild type (WT) TNRC6A and its mutant proteins. (B–E) HeLa cells expressing wild type myc-GFP-TNRC6A or the indicated mutant proteins without or with FLAG/HA-Ago2 were treated with LMB for 4 hr and stained with an anti-HA antibody, followed by Cy5-conjugated anti-rabbit IgG. Fluorescent images are shown from the left: GFP signals of myc-GFP-TNRC6A or its mutant proteins; Cy5 signals of the second antibody against anti-HA antibody; the merged images of GFP and Cy5, in which GFP is shown in green, the Cy5 in magenta; the merged images with DAPI (blue). Bars, 10 µm. (F) The ratio of cells expressing GFP-signal of myc-GFP-TNRC6A or its mutants exclusively in the nucleus (N, blue), cytoplasm (C, red), or both (N+C, yellow).
Supplementary Figure S3.
Overexpression of Ago2 inhibits the nuclear transport of the diffused signals of myc-GFP-TNRC6A by the treatment of LMB. (A) HeLa cells expressing wild type myc-GFP-TNRC6A were treated with and without LMB, and stained with an anti-GFP antibody, followed by FITC-conjugated anti-rabbit IgG. Fluorescent images are shown from the left: FITC signals of the second antibody against anti-GFP antibody; the merged images of FITC and DAPI, in which FITC is shown in green, DAPI in blue. (B) HeLa cells expressing wild type myc-GFP-TNRC6A with FLAG/HA-Ago2 were treated with and without LMB, and stained with an anti-GFP antibody and an anti-HA antibody, followed by FITC-conjugated anti-rabbit IgG and Cy5-conjugated anti-mouse IgG. Fluorescent images are shown from the left: FITC signals of the second antibody against anti-GFP antibody; Cy5 signals of the second antibody against anti-HA antibody; the merged images of FITC and Cy5, in which
FITC is shown in green, the Cy5 in magenta; the merged images with DAPI (blue). Bars, 20 μm. (C) The ratio of cells in which the diffused signals of myc-GFP-TNRC6A-WT in the nucleus were stronger than (N>C, blue), equal to (N=C, yellow), or weaker than (N<C, red) those in the cytoplasm.
Supplementary Figure S4.
Fluorescent microscopy images of the cells shown in Figure 3A. HeLa cells were transfected with pmyc-GFP-TNRC6A-NES-mut and the mixture of different amount of pFLAG/HA-Ago2 and pFLAG/HA-FL (total amount = 0.5 µg). Cells were stained with an anti-HA antibody, followed by Cy5-conjugated anti-mouse IgG. Bars, 20 µm.
Supplementary Figure S5.

Fluorescent microscopy images of the cells shown in Figure 3B. (A) HeLa cells were transfected with 0.5 µg/well of pmyc-GFP-TNRC6A-NES-mut and 0.5 µg/well pFLAG/HA-FL, and stained with an anti-Ago2 antibody, followed by Cy5-conjugated anti-mouse IgG. (B–D) HeLa cells were transfected with 0.5 µg/well of pmyc-GFP-TNRC6A-NES-mut, 0.005 µg/well pFLAG/HA-Ago2 and 0.495 µg/well pFLAG/HA-FL. Among them, fluorescent microscopy images of the cells expressing myc-GFP-TNRC6A-NES-mut exclusively in the cytoplasm (B), in both cytoplasm and nucleus (C), or exclusively in the nucleus (D) were shown. Asterisks indicate the cells in which GFP signals were not detected. Bars, 20 µm.
Supplementary Figure S6.
The amount of TNRC6A-interacting Ago2 protein increases by Ago2 overexpression. HeLa cells expressing myc-GFP-TNRC6A-NES-mut with FLAG/HA-FL or -Ago2 were lysed and immunoprecipitated with an anti-GFP antibody. The immunoprecipitates (IP) and cell lysates (input) were analyzed by western blot using anti-GFP antibody, anti-Ago2, and anti-FLAG antibodies.
Supplementary Figure S7.

TNRC6A is anchored in the P bodies by Ago2 overexpression. (A–C) Fluorescent microscopy images of HeLa cells expressing myc-GFP-TNRC6A-NES-mut and FLAG/HA-Ago2. Cells were stained with an anti-HA antibody (A), an anti-Dcp1 antibody (B) or an anti-RCK/p54 antibody (C). Yellow arrows indicate TNRC6A-NES-mut-positive foci. Bars, 20 µm.
Supplementary Figure S8.
Ago2-Y529E is not localized in the P Bodies. (A) HeLa cells expressing FLAG/HA-Ago2-WT or -Y529E were stained with anti-HA and anti-Dcp1 antibodies, followed by FITC-conjugated
anti-rabbit IgG and Cy5-conjugated anti-mouse IgG. Fluorescent images are shown from the left: FITC signals of the second antibody against anti-HA antibody; Cy5 signals of the second antibody against anti-Dcp1 antibody; the merged images of FITC and Cy5, in which FITC is shown in green, Cy5 in magenta. (B) HeLa cells expressing FLAG/HA-Ago2-WT or -Y529E were stained with anti-HA and anti-RCK/p54 antibodies, followed by FITC-conjugated anti-mouse IgG and Cy5-conjugated anti-rabbit IgG. Fluorescent images are shown from the left: FITC signals of the second antibody against anti-HA antibody; Cy5 signals of the second antibody against anti-RCK/p54 antibody; the merged images of FITC and Cy5, in which FITC is shown in green, Cy5 in magenta. Bars, 20 µm.
Supplementary Figure S9.
Transfection of small RNAs does not affect the subcellular localization of TNRC6A-NES-mut. (A–D) HeLa cells expressing myc-GFP-TNRC6A-NES-mut were transfected with mock (A), siControl (B), miR-200b (C) or let-7b (D). Fluorescent microscopy images of GFP are shown in the left panels, and their merged images with DAPI are shown in the right panels, in which the GFP signals are shown in green and the DAPI signal in blue. Bars, 20 µm. (E) The ratio of cells expressing GFP-signal of myc-GFP-TNRC6A-NES-mut exclusively in the nucleus (N, blue), cytoplasm (C, red), or both (N+C, yellow).
Supplementary Figure S10.
Overexpression Ago2 or TNRC6A does not affect the expression levels of endogenous TNRC6A or Ago2. Western blot with anti-TNRC6A, anti-Ago2 antibodies, and anti-FLAG or anti-GFP antibody was performed using the cell lysates corresponding to Figure 7C or E (250 ng/well of pFLAG/HA, pFLAG/HA-Ago2, pmyc-GFP-TNRC6A-WT, or pmyc-GFP-TNRC6A-NES-mut). Note that HeLa cells express two isoforms of TNRC6A, ~210 kDa TNRC6A and ~182 kDa TNRC6A proteins endogenously, and that the bands of former protein did not separate from the bands of myc-GFP-TNRC6A-WT or -NES-mut. An anti-tubulin antibody was used as a loading control.
Supplementary Figure S11.

Modulation of RNAi and miRNA silencing activities induced by siRNA in the TNRC6A overexpression condition. HeLa cells were transfected with a mixture of the following four plasmids: psiCHECK-CXCR4-PM (A) or -MM 4x (B), pGL3-Control, siCXCR4 or control siGY441 (left panel) or siDsRed (right panel), without or with 2.5, 7.5, or 25 ng/well of pFLAG/HA, pFLAG/HA-Ago2, pmyc-GFP, pmyc-GFP-TNRC6A-WT, or pmyc-GFP-TNRC6A-NES-mut. Two day after transfection, luciferase activities were measured. The relative luciferase activity of siCXCR4-transfected cells against those of siGY441- or siDsRed-transfected cells were set to “1”. The data were shown as the mean and standard deviation of the triplicate measurements. The actual values of the relative luciferase activities of the control cells were 2.1 ± 0.31% (A, left panel), 2.1 ± 0.27% (A, right panel), 3.1 ± 0.38% (B, left panel) and 1.5 ± 0.10% (B, right panel), respectively.