Yao et al Figure S1

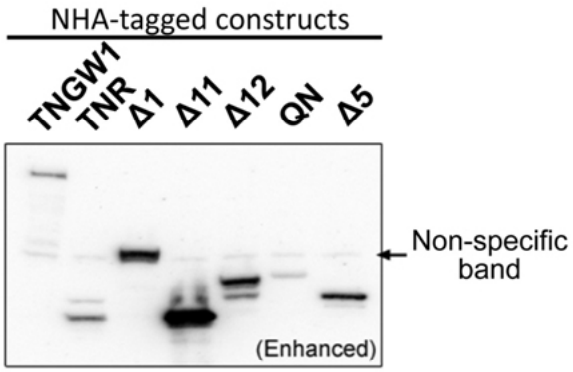

FIGURE S1. The expressions of selected NHA-constructs from representative tethering experiments were monitored by western blot using anti-HA monoclonal antibody. Signals were enhanced by increasing contrast to better visualize the low level of the NHA-TNGW1 band.

Yao et al Figure S2

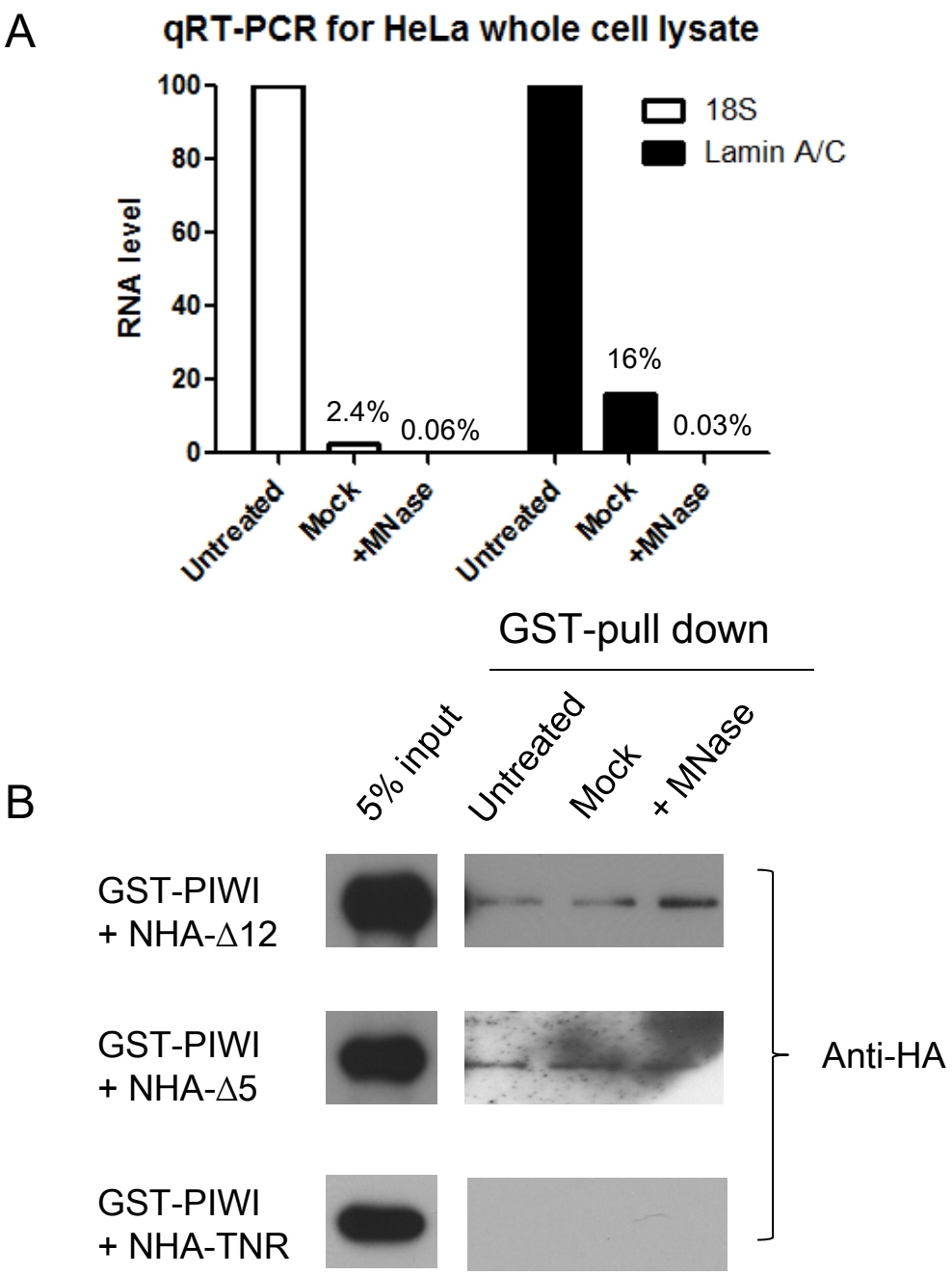

FIGURE S2. The interactions of GW182 repression domains and Ago2 PIWI domain was independent of RNA. (A) The conditions for MNase digestion effectively reduced the RNA level in HeLa whole cell lysates. Note that the incubation conditions for MNase (Mock) already demonstrated significant degradation of RNA. (B) Interactions of Δ12 and Δ5 with PIWI were independent of RNA. NHA-TNR served as negative control.

Yao et al Figure S3

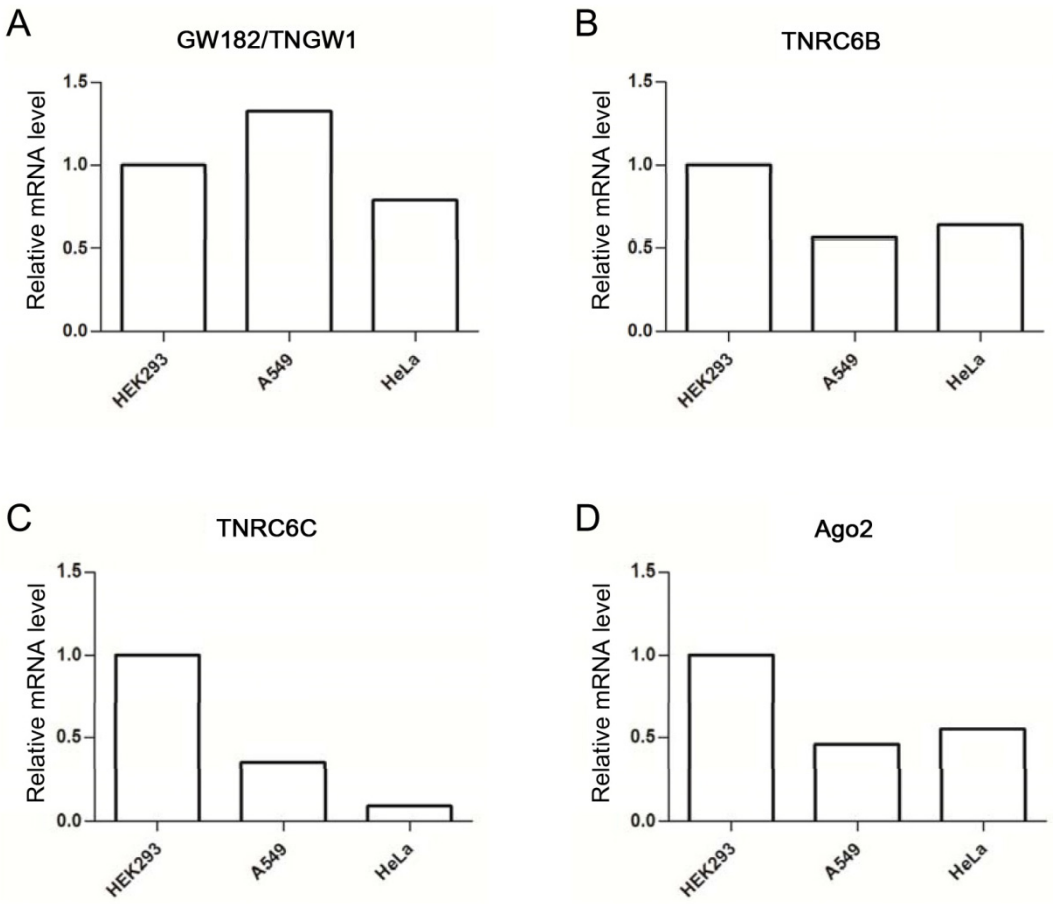

FIGURE S3. TNRC6C expression was very low in HeLa cells compared to A549 and HEK293 cells. To evaluate the relative expression levels of GW182-related protein and Ago2 in different cell lines that were used to perform tethering and RNA interference assay, RNA from each cell line was extracted and examined by qPCR. For comparison, mRNA levels in HEK293 were normalized to 1.

Yao et al Figure S4

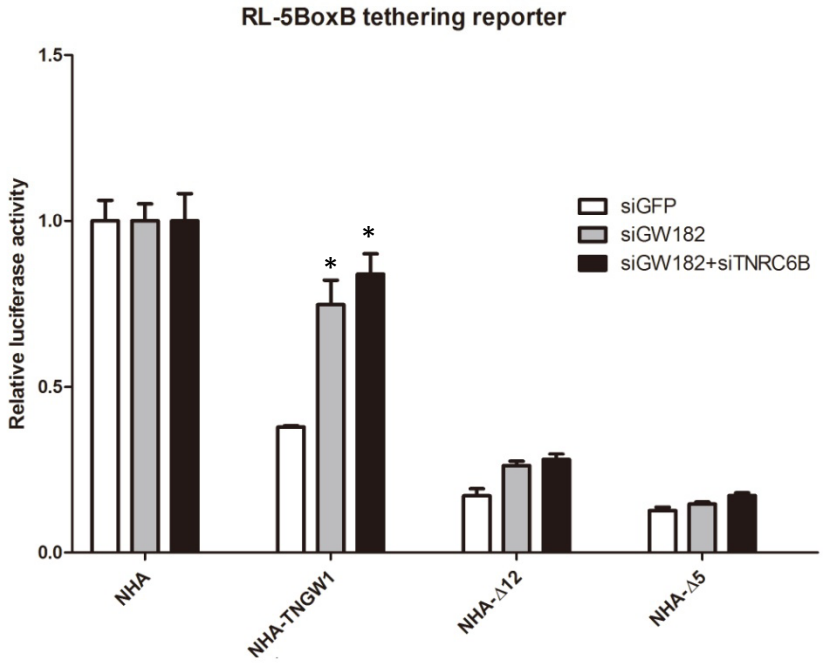

FIGURE S4. Tethered NHA-TNGW1 was employed as an additional control to monitor the GW182 siRNA effects. Repression by Δ5 and Δ12 in tethering assay were independent of endogenous GW182/TNGW1. SiGW182 knockdown impaired NHA-TNGW1 induced tethering repression as expected. In comparison with control siGFP, both siGW182 and the combination of siGW182+siTNRC6B severely impaired the tethered reporter repression indicating the full length TNGW1 was efficiently knockdown. Results are expressed as mean ± standard error from three independent experiments. * represents significant difference in *t*-test compared with siGFP, $p<0.01$.

Yao et al Figure S5

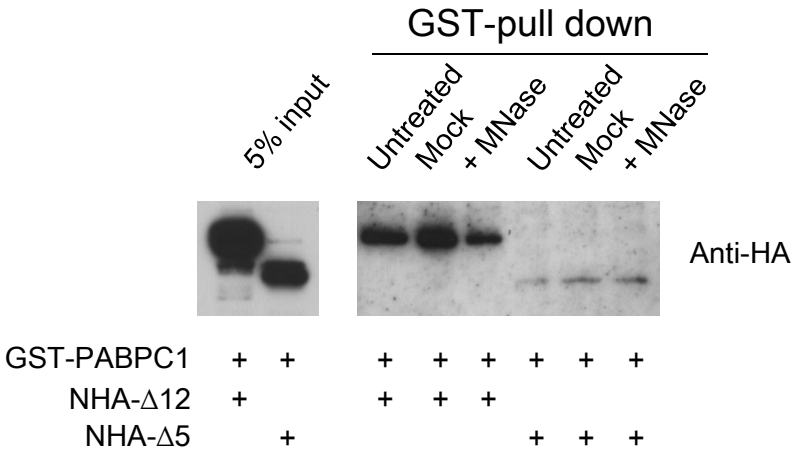

FIGURE S5. The interactions of GW182 repression domains and PABPC1 was independent of RNA. The conditions for MNase digestion were as described in Figure S2.

Yao et al Figure S6

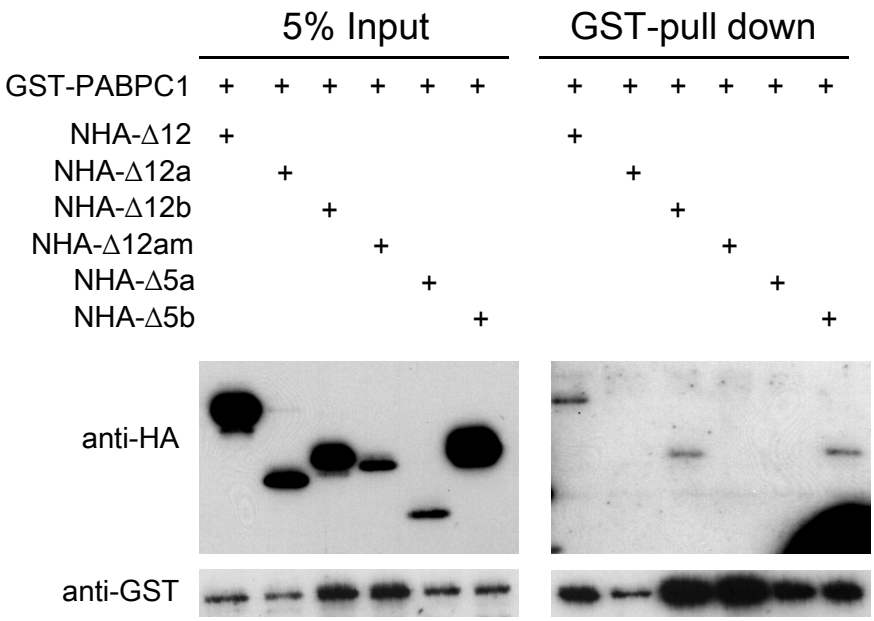

FIGURE S6. Both NHA-Δ12b and NHA-Δ5b bound to GST-PABPC1. GST-PABPC1 was co-transfected with different NHA-tagged constructs into HeLa cells as shown above the panels for the designed GST pull-down assay. After 24 hours, cell lysates were harvested and analyzed by GST pull-down followed by Western blot analysis. NHA-Δ12 served as a positive control as shown in Figure 7.