Supplemental Material and Methods

Plasmid construction

The plasmid expressing β-globulin reporter containing TNF-α UTR was constructed by subcloning β-globulin with TNF-α UTR for pTRE vector to pEF1/Myc-His vector (Invitrogen).

Cell fractionation

HeLa cells were transfected with or without si-TRN for 2 days, followed by transfection with the expression vector encoding FLAG-TTP for 1 day. Cells were harvested after washing with ice-cold phosphate-buffered saline. The cell pellets were resuspended in buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM DTT, proteinase inhibitors (Roche), and 5% glycerol) and incubated on ice for 15 min. Subsequently, Triton X-100 was added to a final concentration of 0.2%, and the lysate was incubated for 2 min at 4°C. After centrifugation at 5000 × *g* for 5 min at 4°C, the cytoplasmic fraction was collected. Nuclear pellets were washed with buffer A and then resuspended in buffer B (20 mM HEPES, pH 7.9, containing 250 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM DTT, proteinase inhibitors and 25% glycerol). After incubation on ice for 30 min and centrifugation at 13000 × *g* for 10 min, the supernatant was collected as the nuclear fraction.

Figure Legends:

Supplemental Figure1: Subcellular localization of TRN in heat-shock or FCCP treated cells. Immunofluorescence was performed using anti-TRN and anti-eIF4A in HeLa cells that were treated at 44˚C for 30 min or with 1 μM FCCP for 90 min. Arrows indicate colocalization of TRN proteins with endogenous SG marker proteins.

Supplemental Figure 2: Subcellular distribution of TRN transport cargoes. HeLa cells were mock-transfected or transfected with TRN siRNA; besides, the FLAG-TTP expression vector was included in all transfectants. The nuclear and cytoplasmic fractions were subjected to immunoblotting using antibodies against hnRNP A1, DDX3 and the FLAG tag (for FLAG-TTP). Lamin A/C and a-tubulin served as markers for the nuclear and cytoplasmic fractions, respectively.

Supplemental Figure 3: Subcellular localization of EDC4 in TRN-depleted cells. HeLa cells were transfected with siRNA against Luciferase or TRN. Immunostaining

was performed using anti-EDC4 antibody. Immunoblotting shows the efficiency of TRN depletion.

Supplemental Figure 4: Association of FLAG-TTP to TRN and ARE containing-mRNAs. HEK293 cells were transfected with FLAG-TTP expression plasmid and TNF-α UTR-containing β-globulin reporter. The cell lysate was subjected to immunoprecipitation using anti-FLAG antibody. The bound proteins were subjected to immunoblotting, followed by RT-PCR analysis of precipitated RNAs.

Supplemental Figure 5: Subcellular localization of GFP-TIA1 with marker for stress granules in TRN depleted-cells. The GFP-TIA1-expressing vector was co-transfected with or without TRN siRNA to HeLa cells. GFP-TIA1 fluorescence was directly detected under microscopy and the endogenous PABP1 was staining using antibody against PABP1. Bar graph: percentage of transfected cells that contained GFP-TIA1-stained SGs. The data was obtained from two independent experiments; ~ 150 transfected cells were counted in each experiment.