SUPPLEMENTARY INFORMATION

Figure Legends:

SFig. 1. Distribution and localization of synthetic tiRNAs in U2OS cells. Immunofluorescence microscopy of U2OS cells transfected with (A) no RNA,, 750 nM of biotin labeled (B) control RNA (P-ctrlRNA) (C) ph3'tiRNA^{Ala} (P-3'Ala) or (D) P-5'tiRNA^{Ala} (P-5'Ala) and stained with SG markers [(anti-G3BP (red) and eIF3b (blue)] at 7 hr post transfection. Biotin labeled RNAs were detected with streptavidin (green). Yellow arrows indicate cells with SGs. White arrows indicate tiRNA.

SFig. 2. Dose dependent effect of synthetic P-5'tiRNA^{Ala} on SG formation in U2OS cells. Immunofluorescence microscopy of U2OS cells transfected with (A) 35 nM, (B) 75 nM, (C) 150 nM, (D) 250 nM, (E) 375 nM, (F) 500 nM, (G) 750 nM, and (H) 1000 nM of synthetic P-5'tiRNA^{Ala} (P-5'Ala) stained with SG markers [anti-eIF4G (green), anti-G3BP (red), and eIF3b (blue)] at 7 hr post transfection. Yellow arrows indicate SG detected. The percentage of cells with SGs are shown for each concentration used.

SFig. 3. Effect of of synthetic small RNAs on SG formation in parental U2OS (A) and stable transfectants expressing recombinant GFP-G3BP/RFP-DCP1a (B), GFP-G3BP (C), GFP-TSN (D), and GFP-ADAR (E). Following transfection, cells were stained with anti-eIF3b and 200 cells were counted to quantify the percentage of cells with SGs.

SFig. 4. Effect of HRI knockdown on SGs formed by P-5'tiRNA^{Ala}. Immunofluorescence microscopy of mock transfected (A and E; No RNA), sodium-arsenite treated (B and F; SA, 200 μ M), pateamine A treated (C and G; PatA, 50 nM), ph5'tiRNA^{Ala} transfected (D and H; ph5'Ala, 750 nM) U2OS cells transfected with control siRNA (D0) (A-D) or with HRI siRNA (HRI) (E-H). The cells were fixed, permeabilized and stained with a PB marker SK1-hedls (green) and SG markers anti-G3BP (red) and anti-eIF3b (blue). Insets are enlarged views showing different colors and merged channels. Yellow arrows indicate representative SGs. White arrow heads indicate PBs.

SFig. 5. Effect of eIF2 α phosphorylation on SGs induced by P-5'tiRNA^{Ala}. Immunofluorescence microscopy showing mock-transfected U20S cells (A and E; No RNA), cells treated with 200 μ M sodium-arsenite (B and F; SA) or 50 nM pateamine A (C and G; PatA), and cells transfected with 750 nM ph5'tiRNA^{Ala} (D and H; ph5'Ala) and stained with anti-HA (green), anti-G3BP (red), and anti- phospho-eIF2 α antibodies (blue). White arrows indicate representative transfected cells overexpressing either wild type eIF2 α (A-D) or non-phosphorylatable mutant of eIF2 α S51A (E-H). Yellow arrows indicate representative SGs.







SFig.3





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