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Supplemental Material to:

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Trafficking of mRNAs containing ALREX-promoting elements through nuclear speckles

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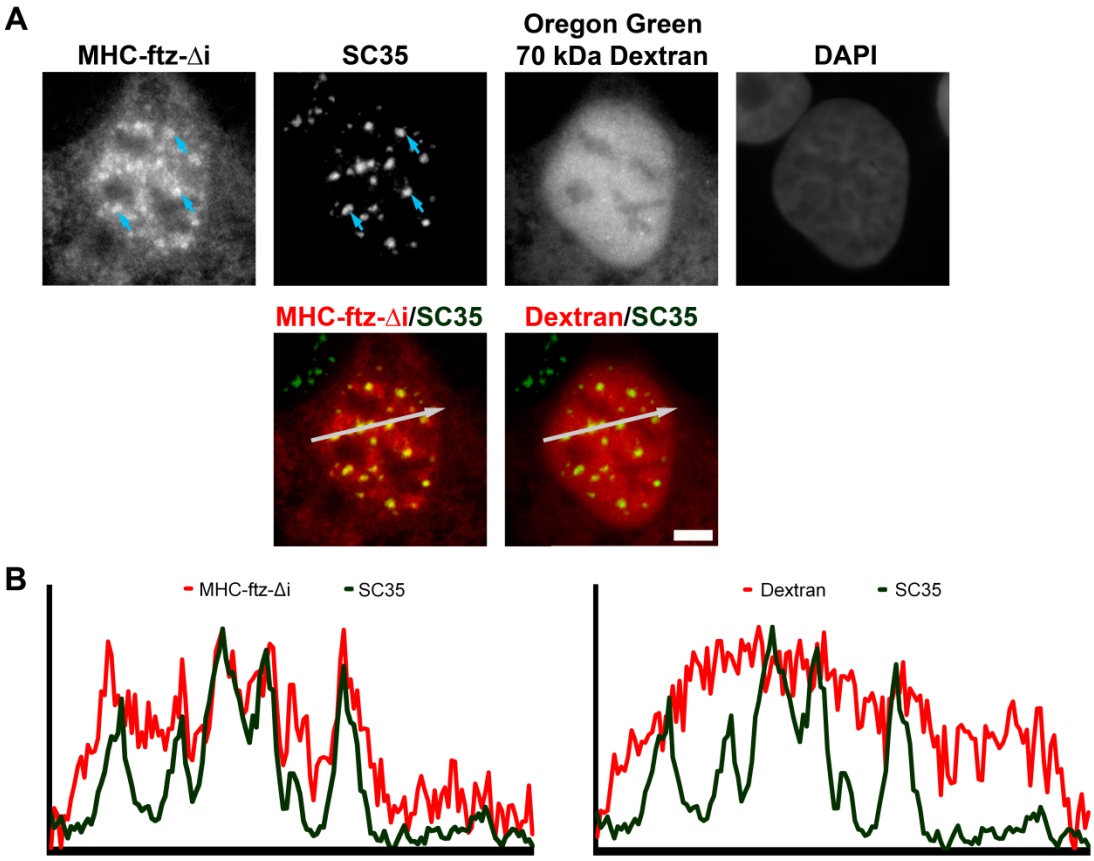
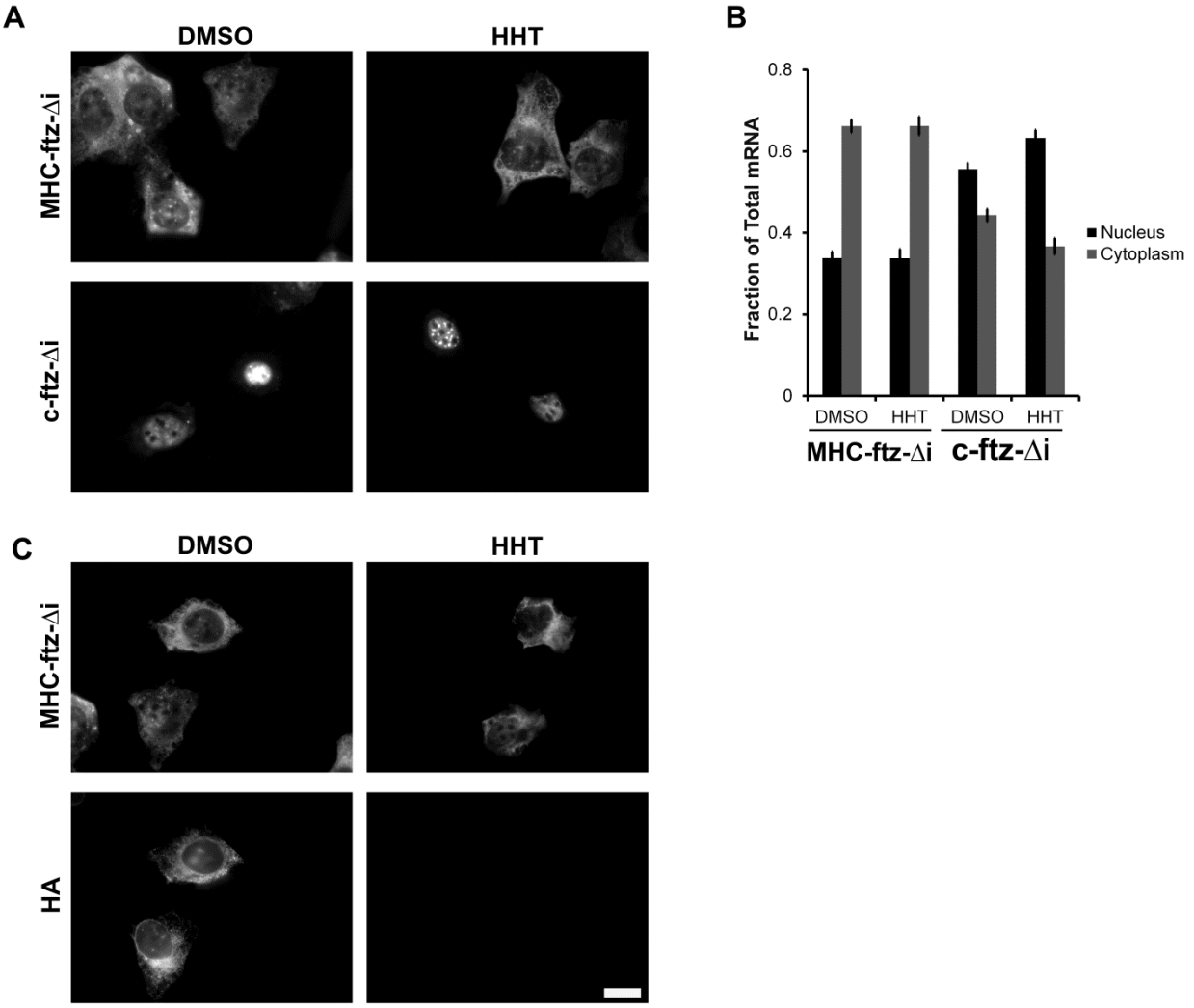
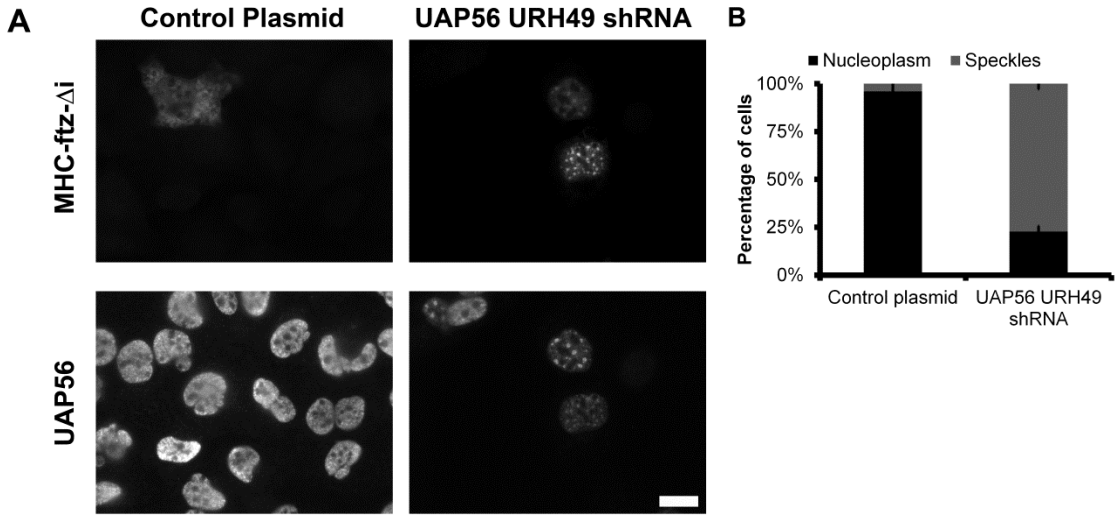


Supplemental Figure 1. *MHC-ftz-Δi* mRNA, but not dextran, colocalizes with SC35-containing nuclear speckles. (A) U2OS were microinjected with DNA plasmid that contains the *MHC-ftz-Δi* construct along with the microinjection marker 70kDa Dextran. After 1 hour, cells were probed for *ftz* mRNA, immunostained for the speckle marker SC35 and stained for DNA using DAPI. All panels are from a single field of view. Overlays of either *MHC-ftz-Δi* or Dextran (red) and SC35 (green) are shown. Scale bar = 5μm. (B) The fluorescence intensities (*y-axis*) of either *MHC-ftz-Δi* or Dextran (red) and SC35 (green) were plotted along the length of the arrow (*x-axis*) as seen in the overlay images in (A).



Supplemental Figure 2. Nuclear export is independent of the translational product of the mRNA. (A-B) U2OS cells were treated with either the translation inhibitor HHT or DMSO for 30 min prior to microinjecting DNA plasmids containing the indicated constructs. After 20min, cells were treated with α-amanitin and mRNA export was allowed to proceed for 2 hours. Cells were then fixed, probed for *ftz* mRNA by FISH, imaged (A) and nuclear export was quantified (B). Each bar represents the average and standard error of three independent experiments, each consisting of 15-60 cells. (C) Cells were treated with either HHT or DMSO for 30 min before plasmids with *MHC-ftz-Δi* were microinjected. Cells were incubated for 2 hours, then fixed,

stained for *MHC-ftz-Δi* mRNA using FISH and *MHC-ftz-Δi* protein using anti-HA antibodies. Each column represents a single field of view. Scale bar = 20μm.



Supplemental Figure 3. Co-depletion of UAP56/URH49 causes the remaining levels of UAP56 to associate with speckles. (A) U2OS cells were treated with lentiviruses to deliver either shRNA directed against UAP56 and URH49 or an empty control plasmid. Cells were then microinjected with plasmids containing *MHC-ftz-Δi.* After 20min, cells were treated with α-amanitin and mRNA export was allowed to proceed for 2 hours. Cells were then fixed, probed for *ftz* mRNA by FISH, and UAP56 by immunofluorescence (A). Each Column is a single field of view. Scale bar = 20μm. (B) Quantification of the percentage of cells showing either nucleoplasmic or speckle distribution for UAP56. Each bar represents the average and standard error of three experiments, each consisting of at least 80 cells.