Supplementary Information for

Title: Distinct roles of hnRNPH1 low-complexity domains in splicing and transcription

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Figures S1 to S7

Supplementary Figure 1

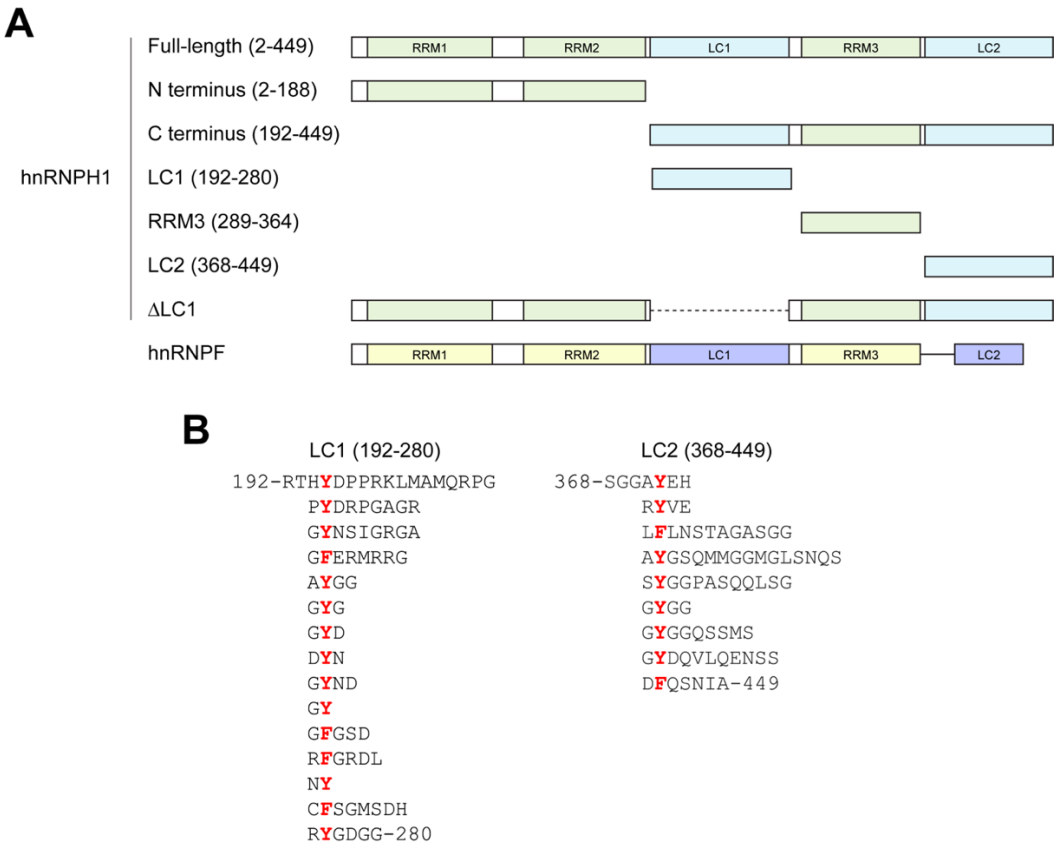

Figure S1. Schematic diagram and amino acid sequence of hnRNPH1. (*A*) Schematic representation of the hnRNPH1 deletion variants and functional domains. (*B*) Amino acid sequences of the LC1 (left) and LC2 (right) domains.

Supplementary Figure 2

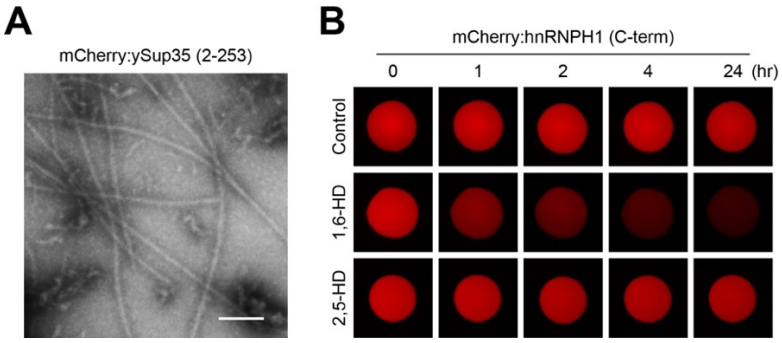

Figure S2. Prion polymers of ySup35 and melting of hnRNPH1 C-terminal hydrogel droplets by hexanediols. (*A*) Polymers composed of mCherry-linked ySup35 recombinant protein was observed by transmission electron microscope (TEM). Scale bar, 0.2 µm. (*B*) Hydrogel droplets composed of mCherry-linked C-terminal half of hnRNPH1 were incubated at 37˚C with 15% levels of 1,6-HD or2,5-HD. The mCherry intensity of the hydrogel droplets were measured by confocal microscopy at indicated time points.

Supplementary Figure 3

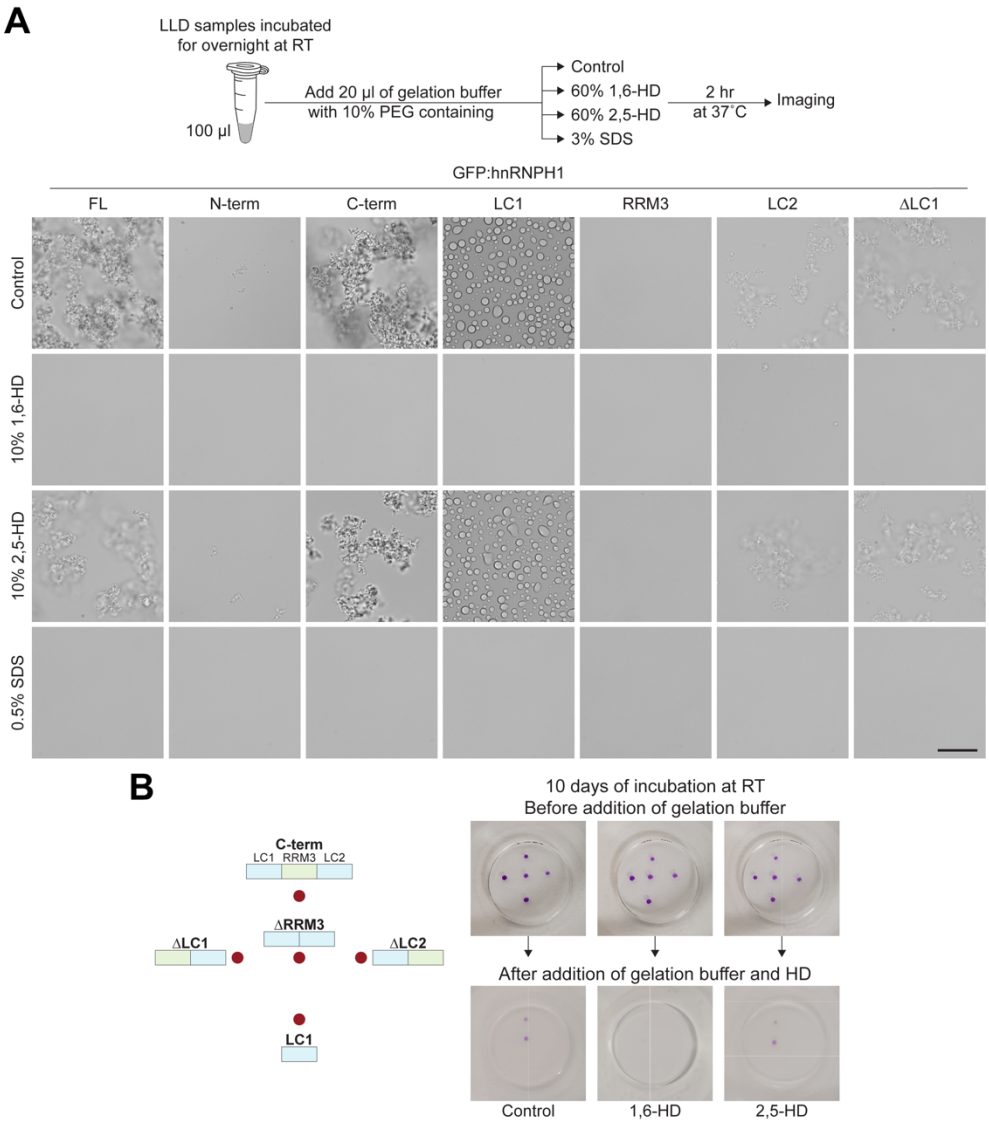

Figure S3. Characterization of hnRNPH1 phase separation. (*A*) Characterization of the aggregation-like precipitates produced from different regions of hnRNPH1. The LLD samples of each protein region was incubated for overnight at room temperature (RT) to produce aggregation-like precipitates as shown in Fig. 2B. Then the LDD samples were supplied with gelation buffer containing 1,6-HD, 2,5-HD, or SDS to reach final concentration of 10% for HDs and 0.5% for SDS. Upon 2 hr incubation at 37˚C, the samples were analyzed using light microscopy. Scale bar, 20 µm. (*B*) Hydrogel droplets of mCherry-linked wild-type C-terminal half (LC1/RRM3/LC2), ΔLC1 (RRM3/LC2), ΔRRM3 (LC1/LC2), ΔLC2 (LC1/RRM3), and LC1 alone of hnRNPH1 were generated onto glass-bottomed confocal dishes. Upon 10 days of incubation at RT, gelation buffer containing 15% 1,6-HD or 2,5-HD were applied to the confocal dishes. After overnight incubation at 37˚C, the formation and melting of the hydrogel droplets were analyzed.

Supplementary Figure 4

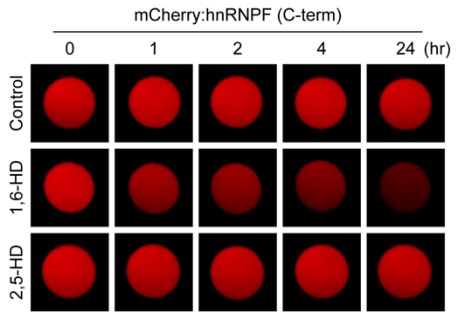

Figure S4. Hexanediol-mediated melting of hydrogel droplets composed of the C-terminal region of hnRNPF. The hydrogel droplets were incubated with 15% levels of indicated hexanediols. Upon incubation at 37˚C for indicated time points, the hydrogel droplets were imaged by confocal microscopy.

Supplementary Figure 5

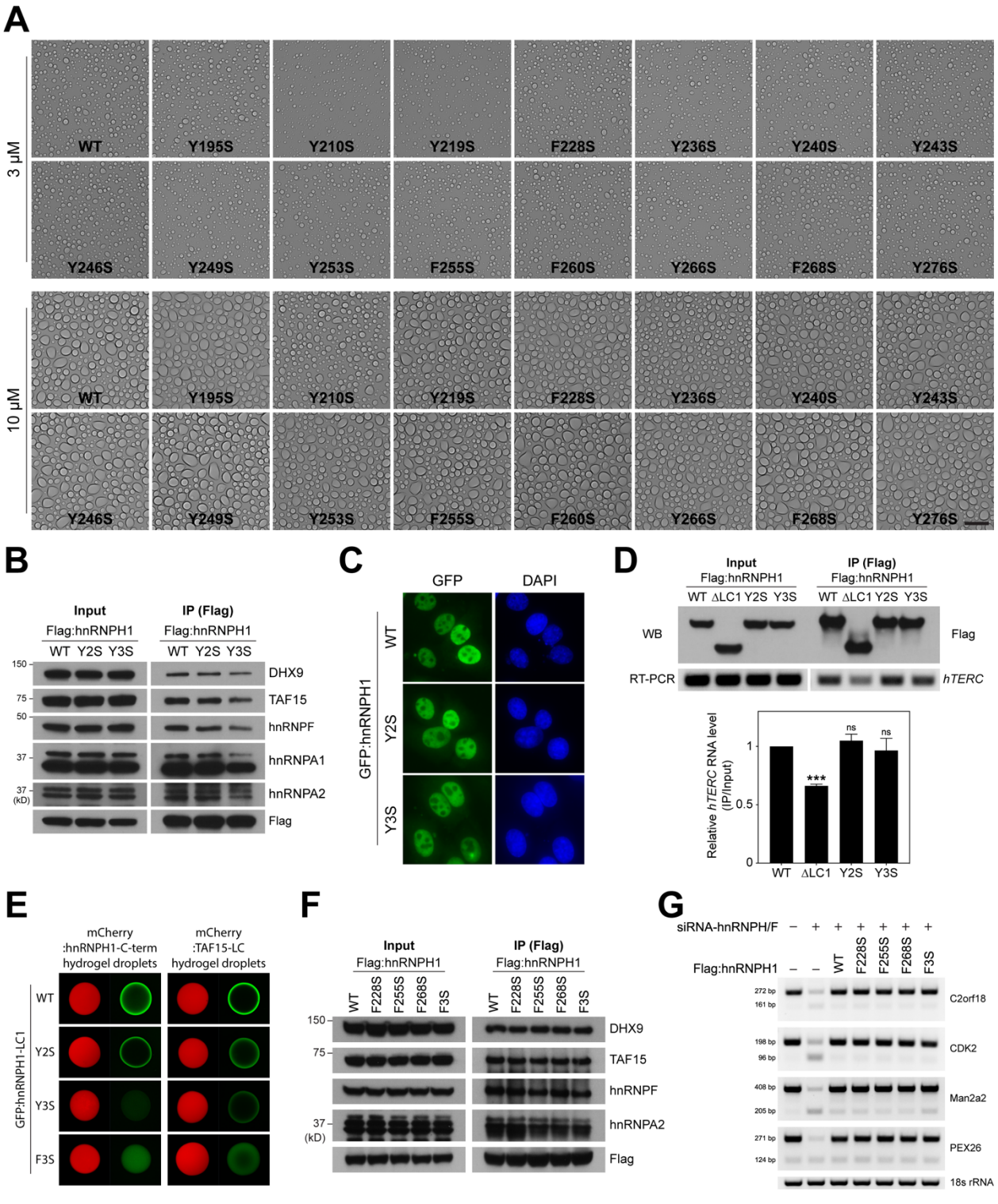

Figure S5. Effects of Y/F-to-S substitutions on phase separation and function of hnRNPH1. (*A*) Formation of LLDs composed of 3 μM (upper panels) and 10 μM (lower panels) of the WT or Y/F-to-S mutant LC1 domain of hnRNPH1. Upon overnight incubation at RT, the LLDs were visualized by light microscopy. Scale bar, 20 μm. (*B*) To analyze the effects of Y2S or Y3S on protein association of hnRNPH1 in living cells, HEK-293T cells were transfected with Flag-tagged wild-type (WT), Y2S, or Y3S hnRNPH1. The cells were then subjected to immunoprecipitation and the interactions between hnRNPH1 and different kinds of RBPs were assessed by Western blotting using indicated antibodies. Representative images are shown from more than three independent immunoprecipitation experiments. (*C*) The effects of Y2S or Y3S on subcellular localization of hnRNPH1 were analyzed. For this, at first, HeLa cells were transfected with GFP-linked WT, Y2S, or Y3S hnRNPH1 full-length. At 48 hr of transfection, the cells were fixed with 4% paraformaldehyde (PFA) and the GFP signals were visualized using fluorescence microscopy (DMi8, Leica, Germany). The DAPI staining was used to visualize the nuclei. (*D*) The effects of ΔLC1, Y2S, or Y3S mutations on RNA binding ability of hnRNPH1 were analyzed by RT-PCR following immunoprecipitation assay. For this, HEK-293T cells were transfected with Flag-tagged WT, ΔLC1, Y2S, or Y3S hnRNPH1. The cells were then subjected to crosslinking and immunoprecipitation (see Materials and Methods). Upon purification of RNAs from both input and IP samples, RT-PCR was performed to analyze the levels of co-precipitated *hTERC* RNA. After agarose gel-running, the band intensity was measured using the program ImageJ (National institute of Health, USA). One-way ANOVA was used to evaluate statistical significance. Mean ± SEM from two independent assays; ns, not significant vs. control; ***$P < 0.0001$ vs. WT. (*E*) Trapping of GFP-linked WT, Y2S, Y3S, and F3S LC1 domains of hnRNPH1 to mCherry hydrogel droplets composed of the C terminus of hnRNPH1 (left) or LC domain of TAF15 (right). (*F*) Pull-down of endogenous proteins by Flag-tagged full-length WT, F228S, F255S, F268S, or F3S hnRNPH1. (*G*) RT-PCR was performed to analyze the effect of F-to-S substitutions of hnRNPH1 on splicing regulation of the indicated transcripts.

Supplementary Figure 6

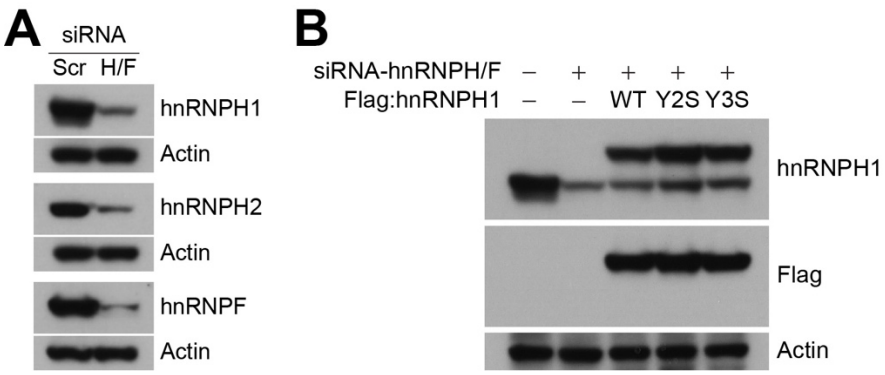

Figure S6. siRNA-mediated Knockdown of hnRNPH1. (*A*) Western blotting was performed to validate knockdown of hnRNPH1/2 and hnRNPF upon transfection of the control siRNA or siRNA targeting hnRNPH and hnRNPF at 10 µM levels. Since the siRNA targeting sequence exists in hnRNPH1 and hnRNPF, but not in hnRNPH2, the reduction in the hnRNPH2 protein levels seem to be a secondary effect; either as a consequence of destabilization of hnRNPF/H1 complexes or hnRNPH2 is a transcriptional target of hnRNPF/H1. (*B*) Transient expression of WT, Y2S, or Y3S hnRNPH1 with N-terminal Flag tags in cells suppressed with the expression of the endogenous hnRNPH1 was analyzed by Western blotting.

Supplementary Figure 7

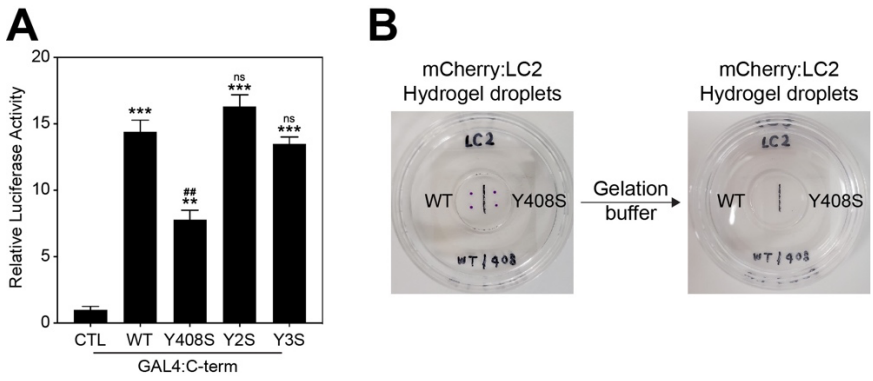

Figure S7. Effect of Y-to-S mutations on transcriptional activity of C-terminal half and phase separation of LC2 domain. (*A*) Transcriptional activity of the wild-type (WT), Y408S, Y2S, or Y3S C-terminal half of hnRNPH1 linked to GAL4 DBD were analyzed in HEK-293T cells. One-way ANOVA was used to evaluate statistical significance. Mean ± SEM from three independent assays; $^{**}P < 0.001$ and $^{***}P < 0.0001$ vs. control; ns, not significant vs. WT; $^{\#\#}P < 0.001$ vs. WT. (*B*) To analyze the effect of Y408S on the hydrogel formation of the LC2 domain of hnRNPH1, hydrogel droplets were generated using recombinant proteins of mCherry-linked WT or Y408S LC2 domains. Neither the WT nor the Y408S LC2 domains became hydrogel droplets until 2 weeks of incubation at room temperature.