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Supplemental information

**Crowding-induced phase separation and gelling by co-condensation of
PEG in NPM1-rRNA condensates**

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Supporting information

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Figure S1

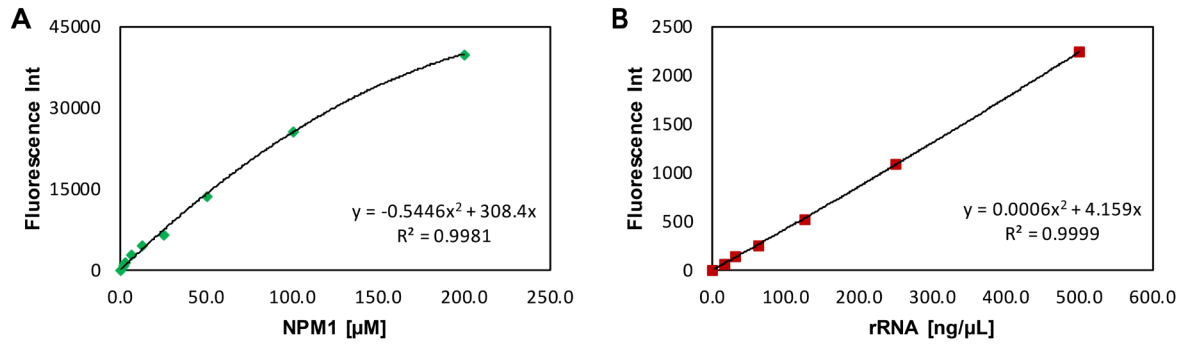


Figure S1: Calibration curves for NPM1 and rRNA. Calibration curves of known concentrations of NPM1 (A) and rRNA (B) that were used to calculate the dilute phase concentrations.

Figure S2

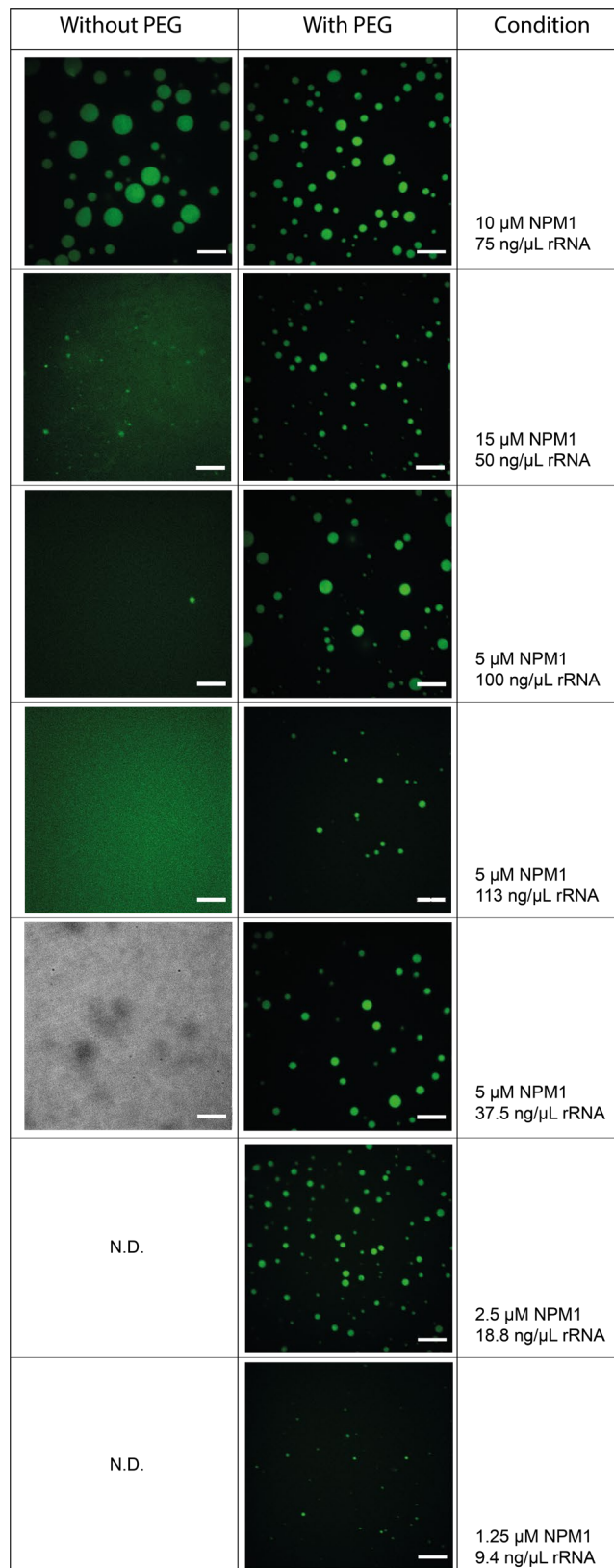


Figure S2: Microscopic assessment of PEG induced phase separation. Varying NPM1 and rRNA concentrations were prepared and examined by fluorescence microscopy for condensation in the absence (0 wt%) or presence (2 wt%) PEG. We defined condensates by the presence of spherical spots with a diameter larger than 2 μm. All scale bars are 10 μm.

Figure S3

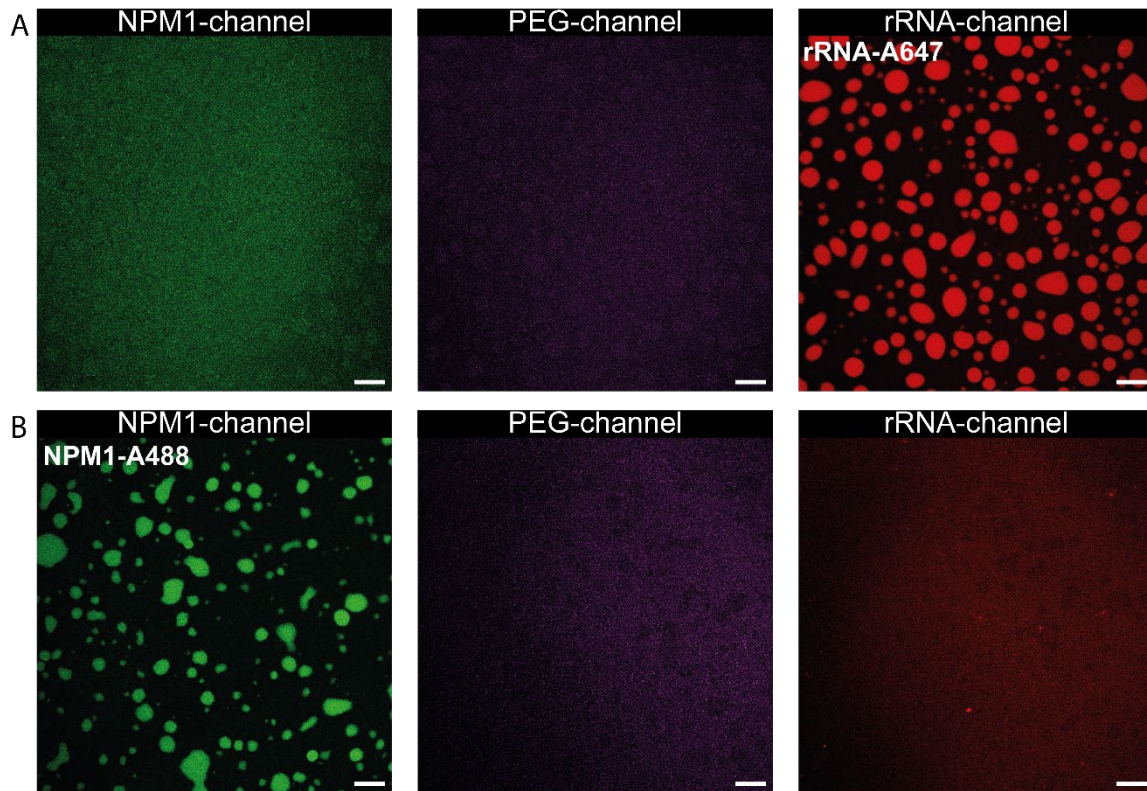


Figure S3: Bleed through experiment of Alexa dyes in the PEG-channel. Control experiment to show the lack of bleed through of the fluorescent dyes from rRNA-A647 and NPM1-A488 in the PEG-channel (573 excitation, 583-649 nm emission filter). (A) NPM1-rRNA condensates with only Alexa647 labelled rRNA. (B) NPM1-rRNA condensates with only Alexa488 labelled NPM1. All scale bars are 10 μm.

Figure S4

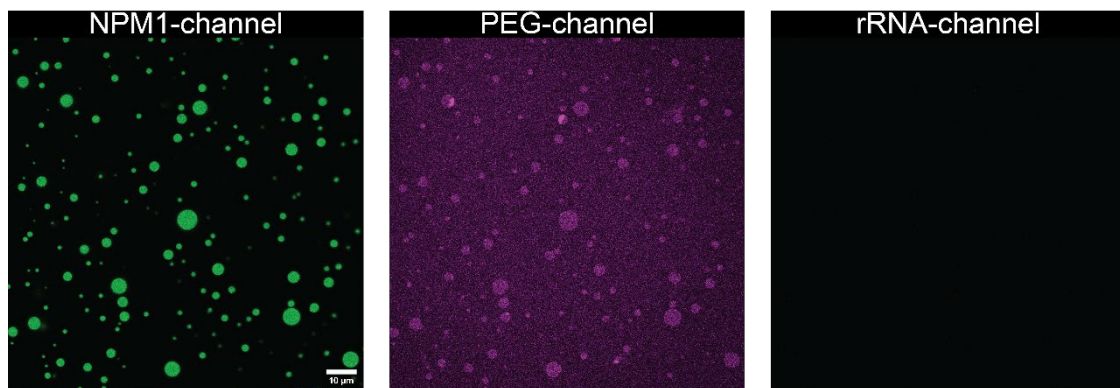


Figure S4: Homotypic NPM1 droplets. Fluorescent microscopy images of NPM1-NPM1 droplets in the presence of 5 wt% PEG-rhodamine. These droplets are characterized by a partitioning coefficient (K_p) of 50 for NPM1-A488, and a K_p of 2 for PEG-rhodamine. Scale bar is 10 μm.

Figure S5

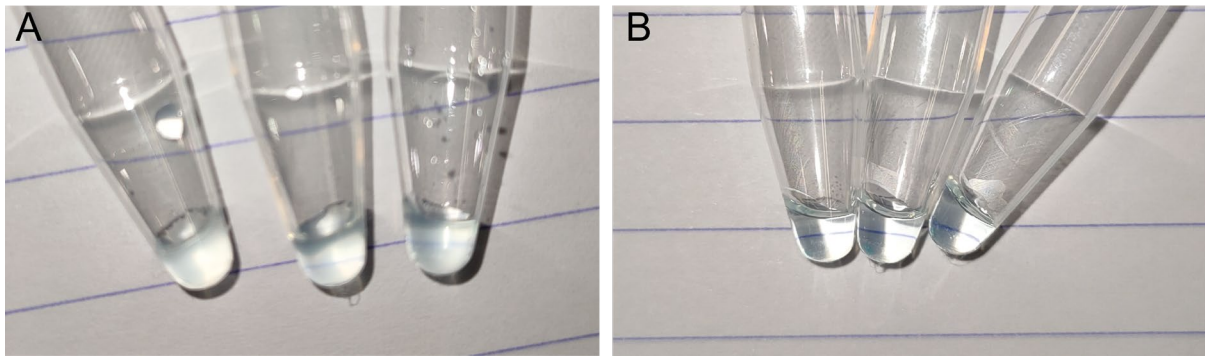


Figure S5: Separation of dilute and condensed phase. (A) Pictures showing turbid suspensions of NPM1-rRNA condensates before centrifugation and (B) clarified solutions with a tiny pellet of condensed phase after 20 minutes of centrifugation.

Figure S6

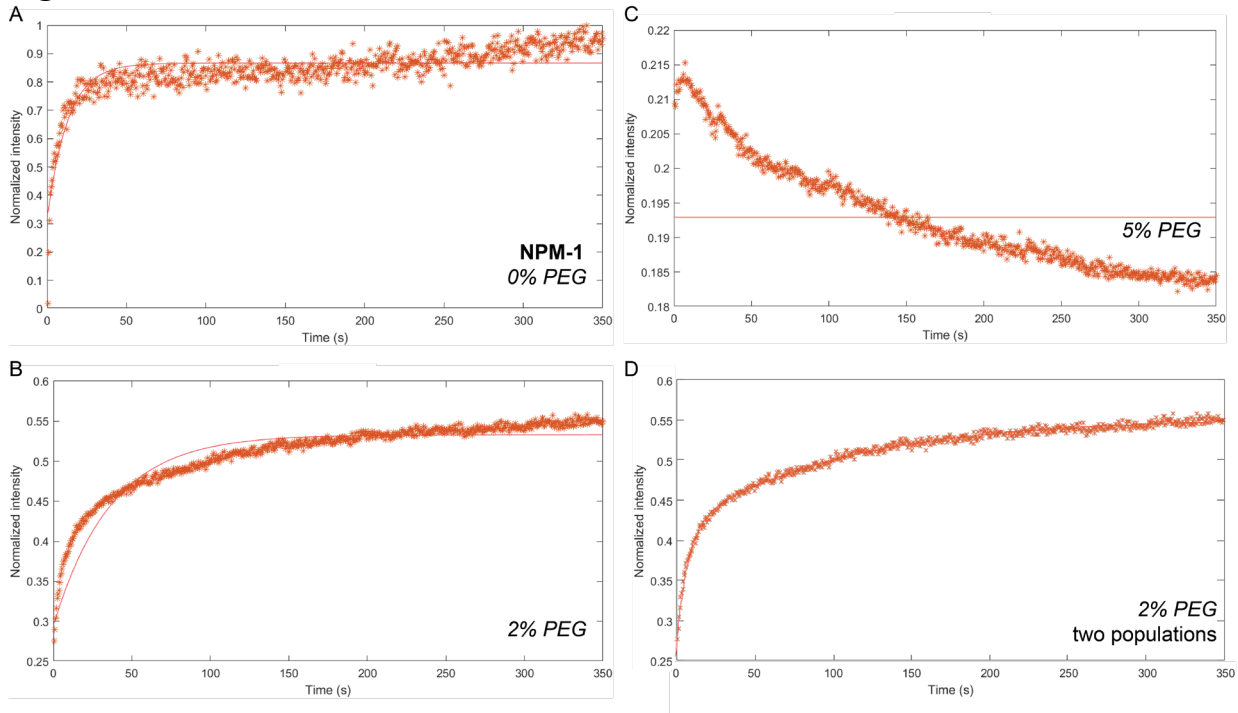


Figure S6: Curve fitting for the fluorescence recovery after photobleaching of NPM1-A488. Zoomed in curve fitting for the FRAP data of NPM1 in the presence of (A) 0% PEG, (B) 2% PEG, (C) 5% PEG, (D) 2% PEG fitted with two populations. For 5% PEG (C) instead of recovery, a slow bleaching of the spot caused by the imaging was observed. The recovery half times ($t_{1/2}$ of the fit in D are 5 s and 71 s, respectively).

Figure S7

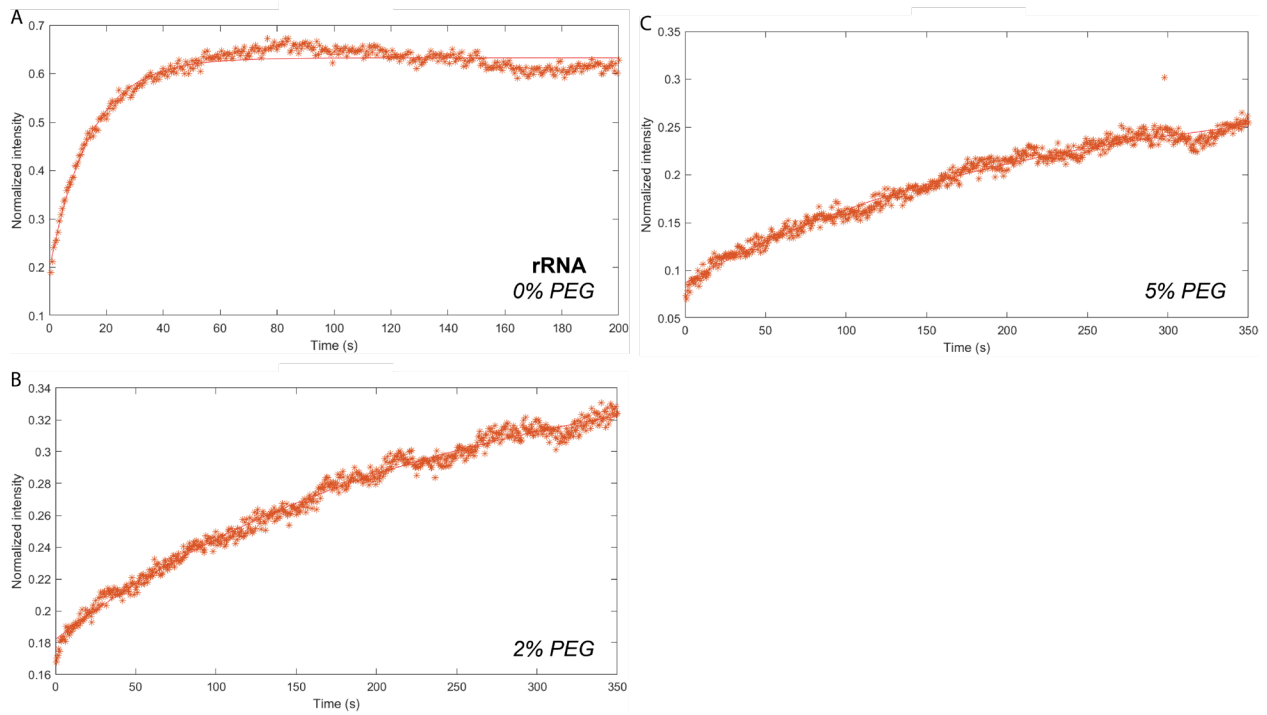


Figure S7: Curve fitting for the fluorescence recovery after photobleaching of rRNA-A647. Zoomed in curve fitting for the FRAP data of rRNA in the presence of (A) 0% PEG, (B) 2% PEG, (C) 5% PEG.