

Supplementary materials

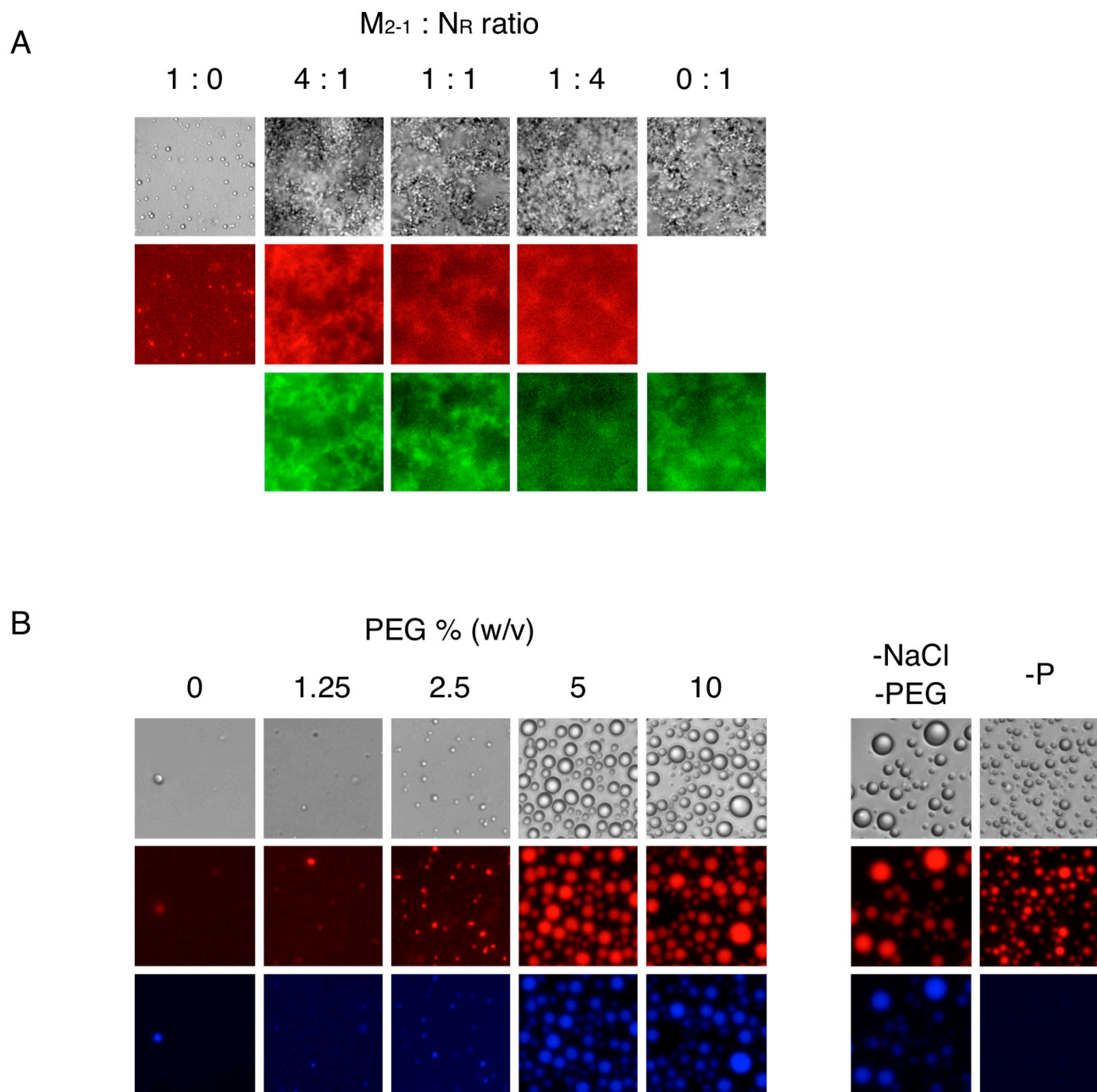


Figure S1. Heterotypic condensation of M_{2-1} and P or N_R . (A) N_R and M_{2-1} were incubated at different ratios with protein concentration ranging from 2.5 to 10 μ M. (B) Effect of crowding agent on heterotypic LLPS. P and M_{2-1} at 1:4 ratio were incubated in the presence of increasing concentrations of PEG-4000. -NaCl -PEG, corresponds to heterotypic P- M_{2-1} condensates without NaCl and crowding agent. -P corresponds to homotypic M_{2-1} condensates with fixed NaCl and PEG-4000.

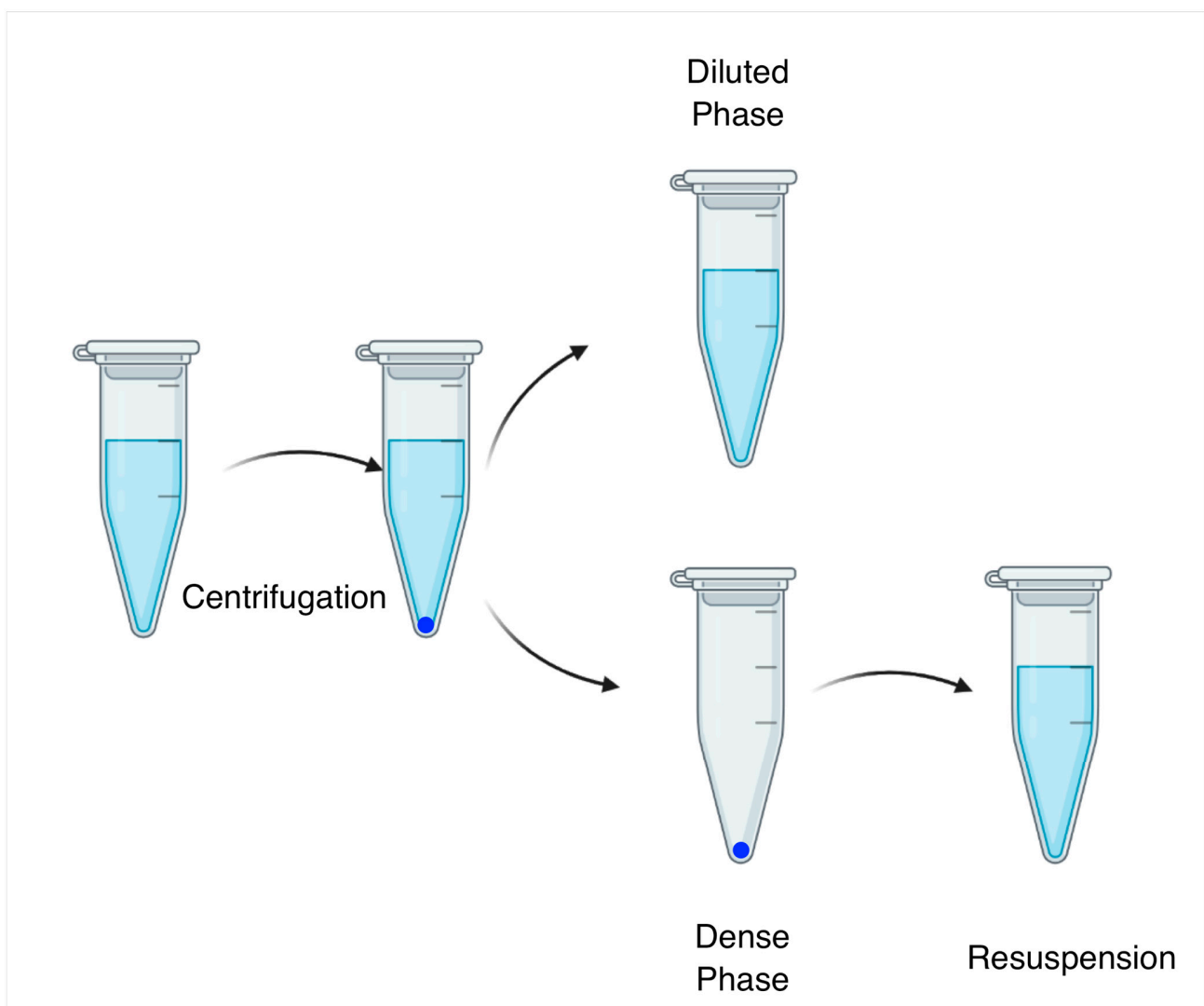


Figure S2. Scheme of partition experiment protocol.

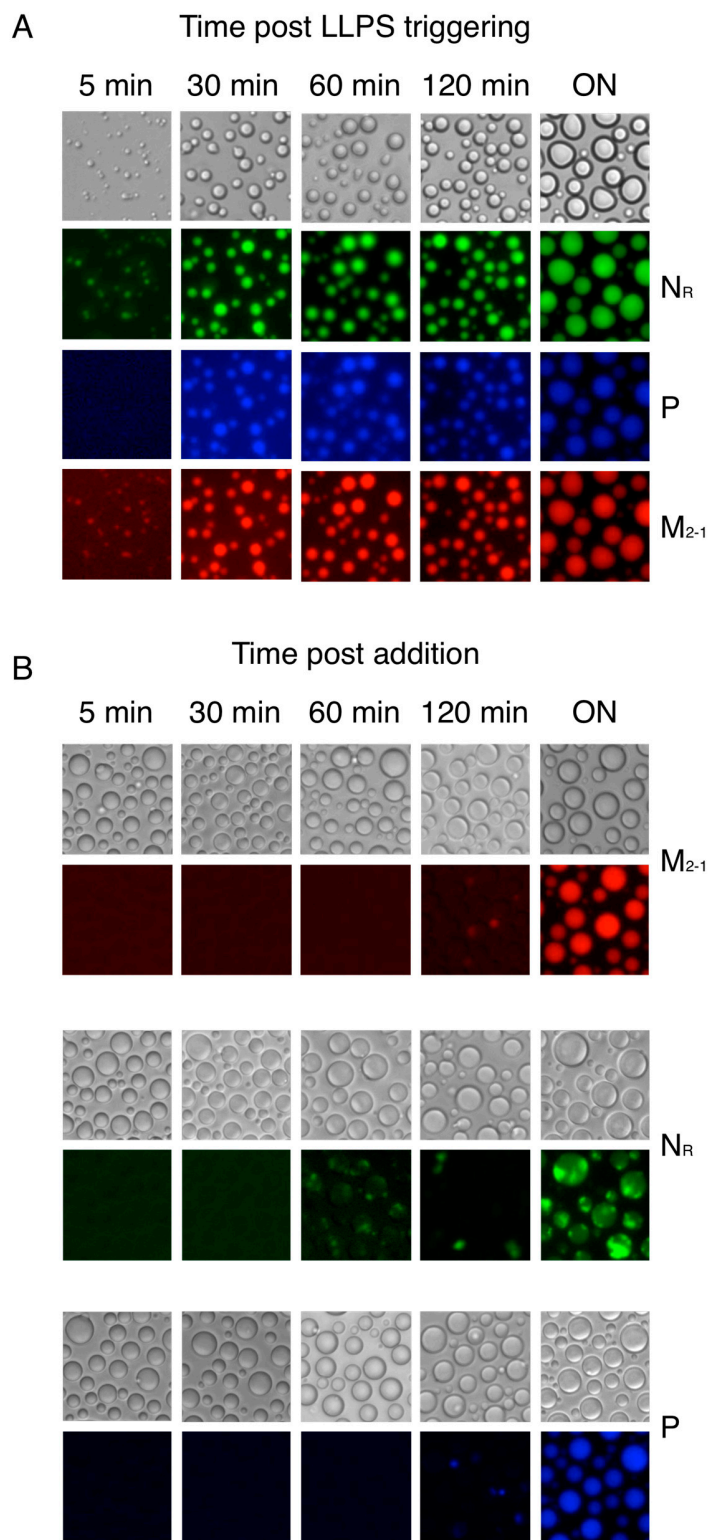


Figure S3. Tripartite P- N_R - M_{2-1} condensates. (A) Formation of tripartite heterotypic condensates triggered by N_R . Preformed P- M_{2-1} soluble complex incubated for 10 min and then N_R was added. (B) Incorporation of M_{2-1} -FITC, N_R -FITC or P-FITC to tripartite condensates of unlabeled P- N_R - M_{2-1} .

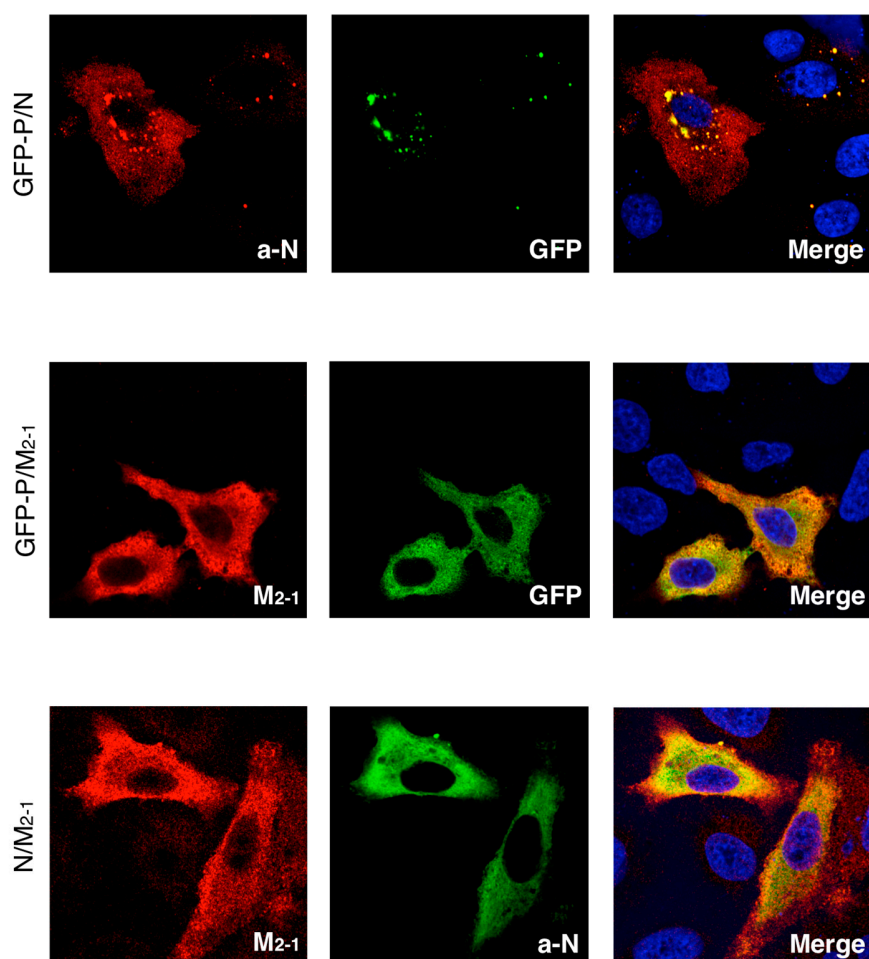


Figure S4. Co-localization of RSV proteins in transfected cells. A549 cells were co-transfected in pairs with plasmids encoding GFP-P, M₂₋₁ and N for 24h as a control. Proteins were detected by fluorescence microscopy labeling by GFP fluorescence or with anti-M₂₋₁ or anti-N antibodies. Nuclei were stained with DAPI.

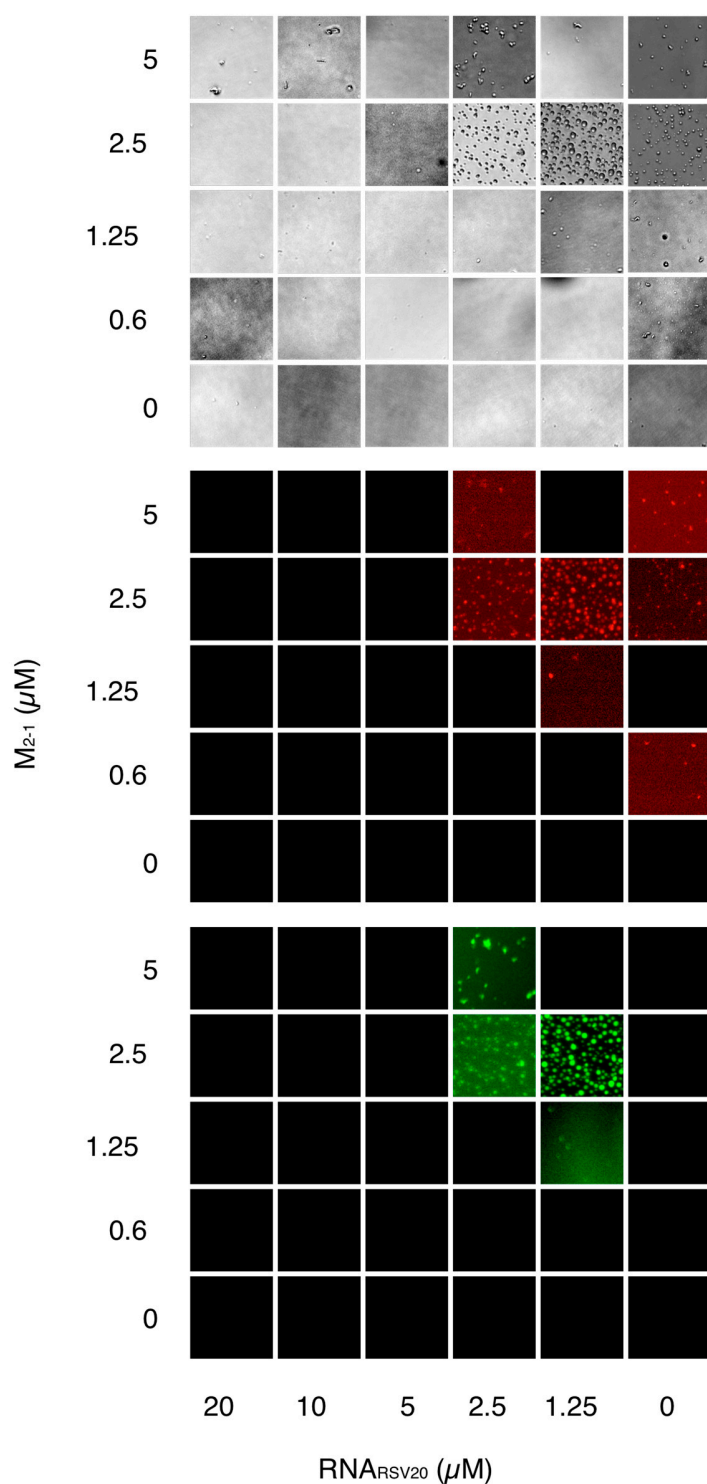


Figure S5. Grid of heterotypic M_{2-1} - RNA_{RSV20} condensates. Different concentrations of M_{2-1} and RSV_{RNA20} were visualized by brightfield microscopy (top) and fluorescence microscopy of M_{2-1} -Cy5 and RNA_{RSV20} -FITC (middle and bottom, respectively).

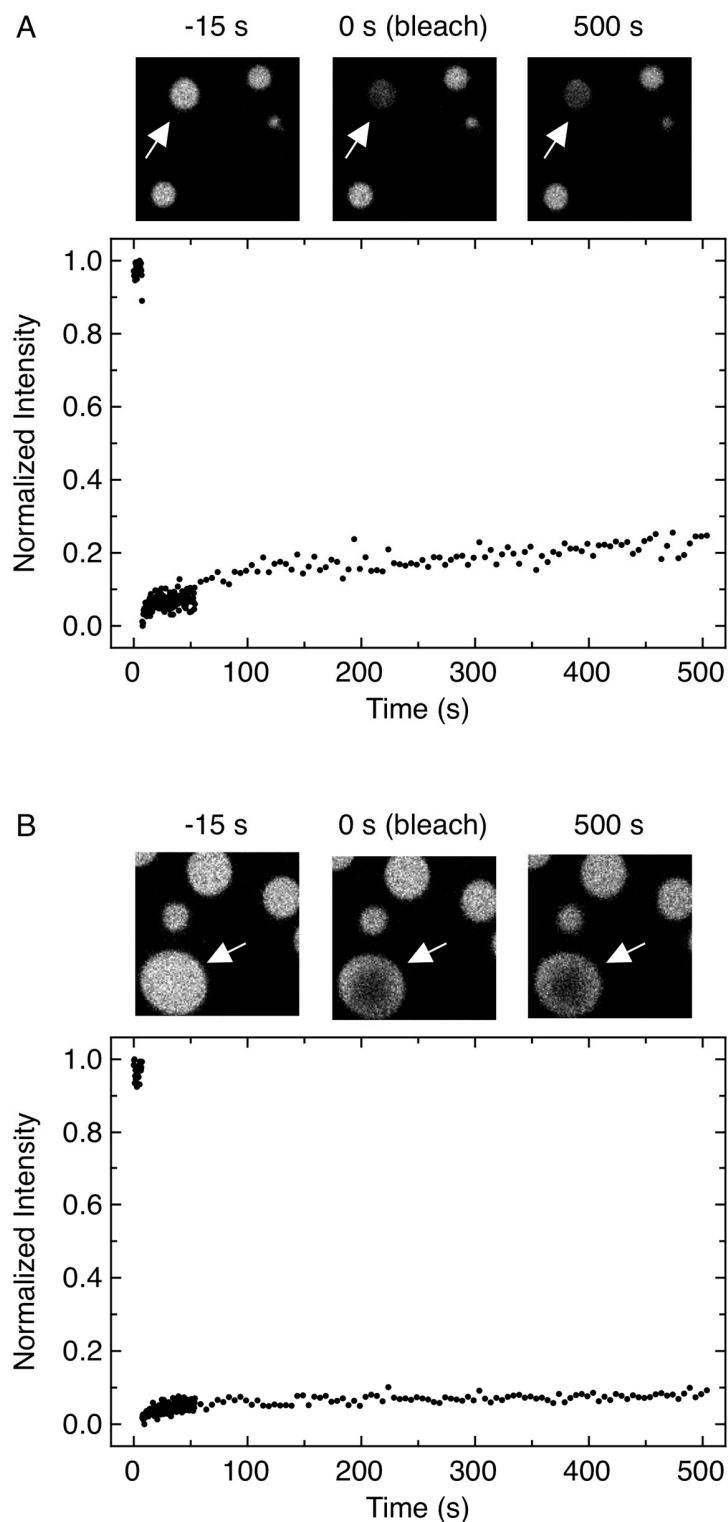


Figure S6. Fluorescence recovery after photobleaching (FRAP) assays of homotypic M_{2-1} and heterotypic M_{2-1} -tRNA₇₀ condensates. (A) Representative images and plot of FRAP analysis performed on FITC- M_{2-1} (5 μ M) homotypic condensates. (B) Representative images and plot of FRAP analysis performed on FITC- M_{2-1} (5 μ M) and tRNA₇₀ (300 mM) heterotypic condensates.

Table S1. Initial and final (condensed and diluted) protein concentration of heterotypic condensates after partition experiments.

	PEG-4000 (% w/v)	Initial mix	Concentration (μM) ¹ Dense phase	Diluted phase
P : M ₂₋₁ (4 : 1)	10	10 : 2.5	195 : 12	6.2 : 2.3
P : M ₂₋₁ (1 : 1)	10	2.5 : 2.5	53 : 24	1.5 : 2
P : M ₂₋₁ (1 : 4)	10	2.5 : 10	70 : 140	1.1 : 7
P : N _R (2.5 : 1)	5	5 : 2	98.3 : 81.3	3 : 0.4
P : N _R : M ₂₋₁ (2.5 : 1 : 2.5)	5	5 : 5 : 2	120.92 : 93.38 : 64.58	2.6 : 0.1 : 3.7

¹ Refers to P tetramer, M₂₋₁ tetramer and N_R decamer (see text).