Supplementary Figures

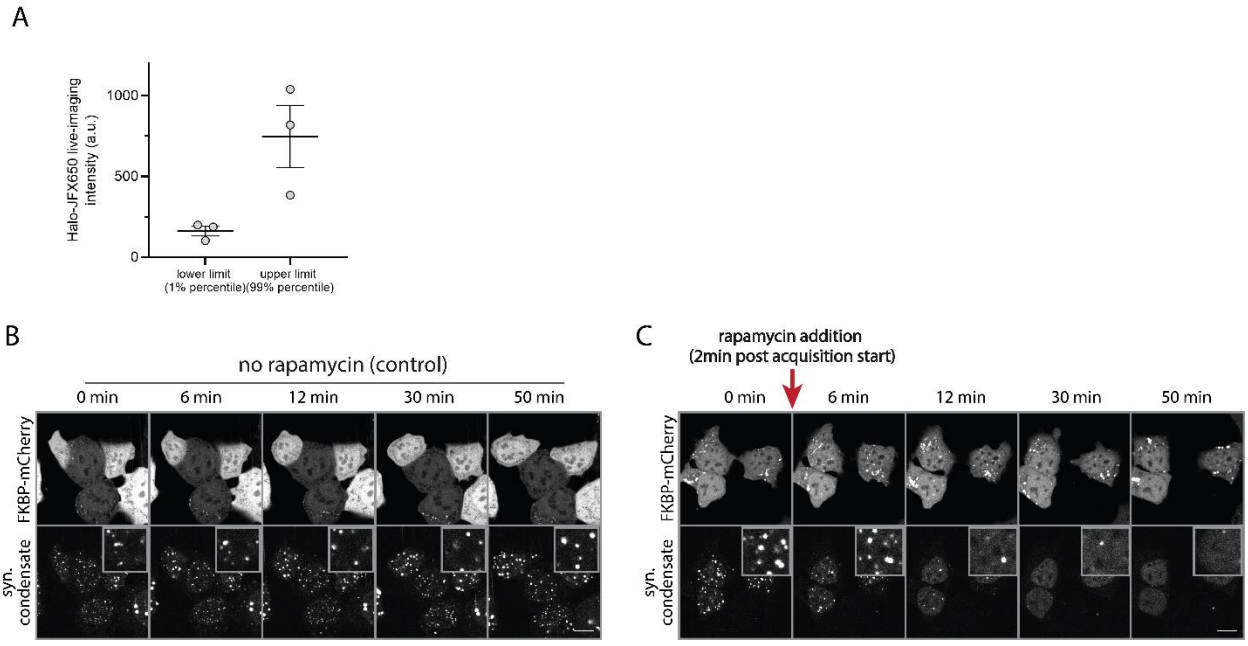

Figure S1 Verification of mCherry as solubility tag

A) Quantification of the endogenous YAP expression range (1-99% percentile) in the Halo-YAP (JFX650) re-expression system. The Halo-tag (JFX650) cells were IF stained for YAP and compared to IF stains of WT cells. Shown is the Halo-YAP (JFX650) intensity (y-axis) corresponding to the lower 1% percentile and upper 99% percentile of the WT distributions (x-axis). Shown are mean +/- SEM from N = 3 independent experiments. **B-C**) Verification of mCherry as a solubility tag in mESCs using the synthetic condensate system (SPARK-ON). Cells expressing the SPARK-ON components and FKBP-mCherry (top row). Pre-formed synthetic condensates (bottom row) were left untreated (B) or acutely treated with rapamycin at 2 min post-acquisition start (B). Note the dissolution of condensates upon mCherry recruitment (inset, bottom row, C) as compared to the control (inset, bottom row, B). Scale bar: 10 µm

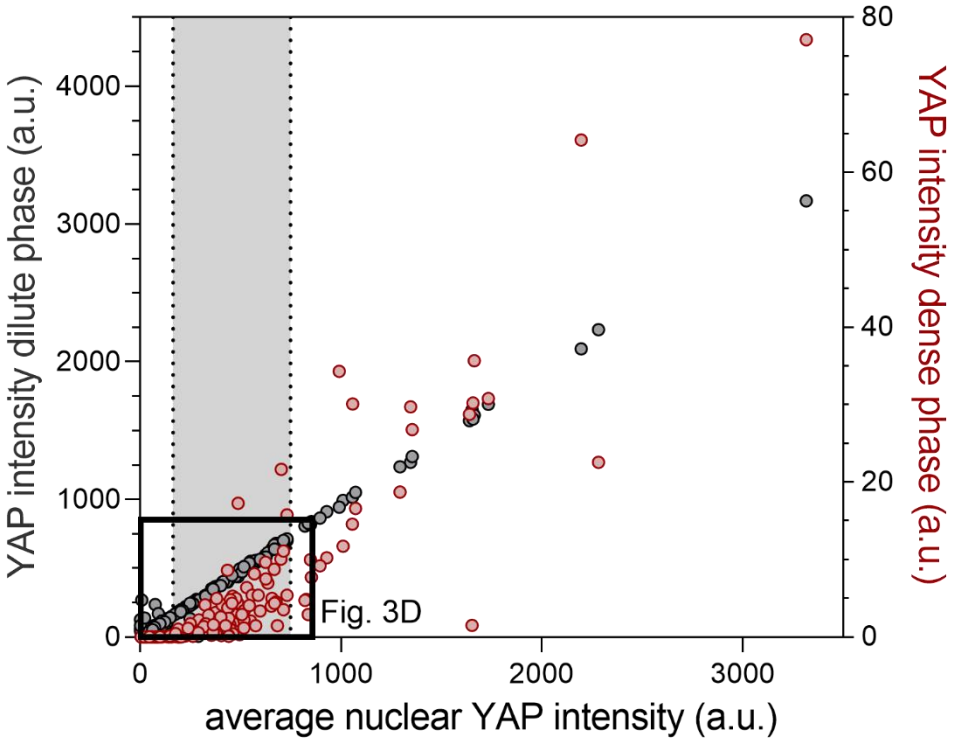

Figure S2 Extended dataset for dense and dilute phase YAP intensities

Full dataset of data shown in Fig. 3D (indicated by black rectangle). For details see Figure legend of Fig. 3D.

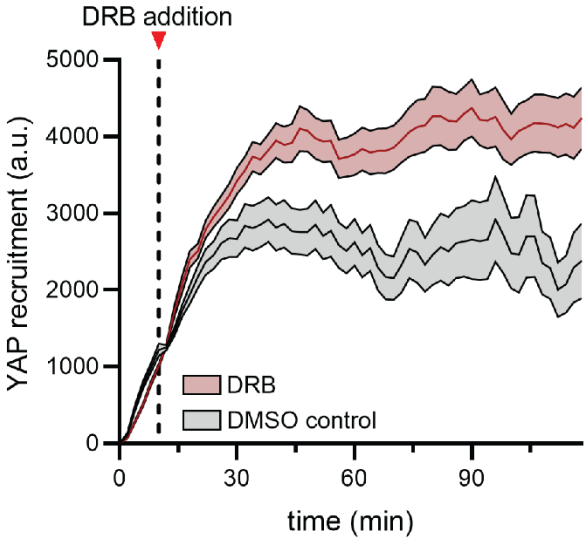

Figure S3 Quantification of YAP recruitment to synthetic condensates upon inhibition of RNA synthesis

Quantitation of YAP recruitment to synthetic condensates following inhibition of RNA synthesis with DRB at t = 12 min (dashed line). DMSO serves as the control. Shown are mean +/- SEM from pooled time courses of N = 4 independent experiments.

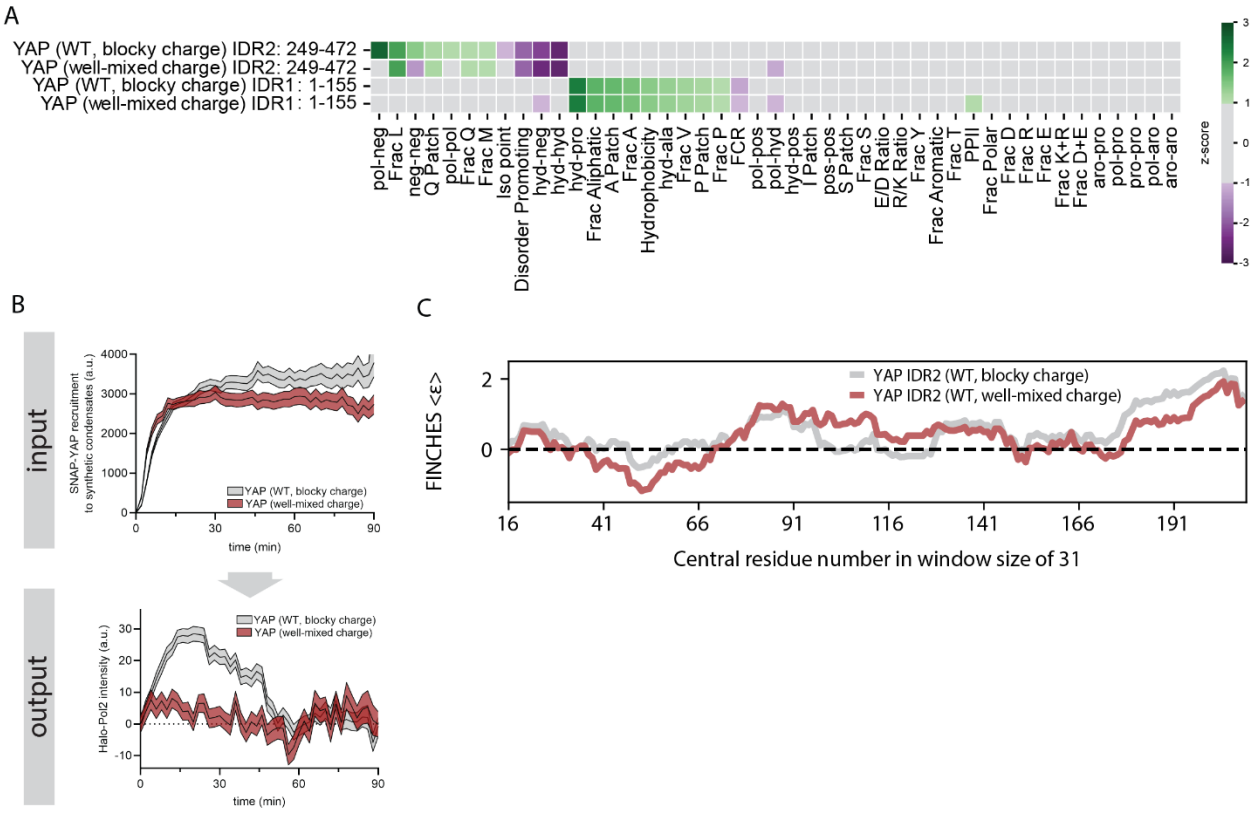

Figure S4 IDR grammar analysis for well-mixed charge YAP variant

A) IDR grammar analysis for sequence features across the YAP IDRs 1 and 2 for the WT and well-mixed charge variant. See IDR Grammar Key in Fig. 6B for features analyzed. **B)** Endogenous Pol2 recruitment to acute YAP condensate formation using the synthetic condensate system (see Fig. 4A), comparing the WT blocky-charge protein with the engineered well-mixed charge variant. Top (input): Recruitment of SNAP-YAP variants (WT blocky charge vs well-mixed charge) to synthetic condensates. Bottom (output): Endogenous Halo-Pol2 response to the acute YAP condensate recruitment (top graph). Shown are mean +/- SEM of pooled time series from N = 4 independent experiments. **C**) Predicted intermolecular interaction maps for the Pol2 IDR and YAP IDR2 of the WT (blocky charge; grey line) and engineered well-mixed charge YAP variant (red line). Epsilon (y-axis) represents the predicted attracting (negative epsilon) and repelling (positive epsilon) interaction based on a number of physio-chemical protein features. Note that the attractive interaction with Pol2 (negative epsilon for residues ~40-70) is predicted to be slightly enhanced in the well-mixed charge YAP variant.