



Supplementary information, Fig. S11 Condensed Ddx3xb is the active form of helicase activity. **a**, *In vitro* helicase assays of purified Ddx3xb_{FL} (100 nM) and Ddx3xb_{ΔN} (100 nM) proteins. **b**, *In vitro* helicase assays of purified Ddx3xb_{ΔN}-hnRNPA1_{IDR} (100 nM), and Ddx3xb_{ΔN}-FUS_{IDR} (100 nM) proteins. **c**, *In vitro* helicase assays of purified Ddx3xb_{ΔN}-YAP_{IDR} (100 nM) proteins. **d,e**, *In vitro* helicase assays using different doses (0 nM, 100 nM, and 200 nM) of purified Ddx3xb_{FL}, Ddx3xb_{ΔN} and Ddx3xb_{ΔN}-YAP_{IDR} proteins. The unwinding ratio was calculated by (unwound RNA) / ((unwound RNA) + (RNA duplex)), Error bars, mean ± SD (n = 3). *P* values

were determined by the two-tailed Student's *t* test, **, $P < 0.01$; ***, $P < 0.001$. **f**, The condensation state of the condensed phase and the dilute phase of Alexa-488 labeled Ddx3xb. Scale bars, 10 μm . **g**, Quantification of condensed fraction of the condensed phase and the dilute phase as observed in experiments shown in (f). $n = 20$ fields per condition. Error bars, mean \pm SD, P values were determined by the two-tailed Student's *t* test, ***, $P < 0.001$. **h,i**, In vitro helicase assays using the same volume of condensed phase and dilute phase. These different phases were separated from purified Ddx3xb proteins (2 μM) by sedimentation assay. The unwinding ratio was calculated by $(\text{unwound RNA}) / ((\text{unwound RNA}) + (\text{RNA duplex}))$, Error bars, mean \pm SD ($n = 3$). P values were determined by the two-tailed Student's *t* test, ***, $P < 0.001$. **j**, The protein concentration of condensed phase and dilute phase. $n = 3$. Error bars, mean \pm SD, P value was determined by the two-tailed Student's *t* test, ***, $P < 0.001$. **k**, The condensation state of the condensed phase (diluted) group and dilute phase group was examined by using Alexa-488 labeled Ddx3xb after 20 min of reaction. Scale bars, 10 μm . **l**, Quantification of condensed fraction of the condensed phase (diluted) group and the dilute phase group as observed in experiments shown in (k). $n = 20$ fields per condition. Error bars, mean \pm SD, P value was determined by the two-tailed Student's *t* test, ***, $P < 0.001$. **m**, The helicase activity of the condensed phase (diluted) group and dilute phase group was examined by *in vitro* helicase assays. The same protein amounts of each groups were used. **n**, The unwinding ratio was calculated by $(\text{unwound RNA}) / ((\text{unwound RNA}) + (\text{RNA duplex}))$, Error bars, mean \pm SD ($n = 3$). P value was determined by the two-tailed Student's *t* test, **, $P < 0.01$. **o**, A time-course assay monitoring the condensation state of condensed phase (diluted) by using Alexa-488 labeled Ddx3xb. Scale bars, 10 μm . **p**, Quantification of condensed fraction of the condensed phase (diluted) group observed in experiments shown in (o). $n = 20$ fields per condition. Error bars, mean \pm SD, P value was determined by the two-tailed Student's *t* test, ***, $P < 0.001$.