a

b

Ddx3xb

0 min   15 min   30 min

c

	-20 s	0 s	60 s	180 s	300 s
RNA					
Ddx3xb					
Merge					

SYTO 64-stained RNA

d

0 mM AMP-PNP   5 mM AMP-PNP

-RNA

+RNA

e

n.s.   ***   ***

0 mM AMP-PNP (-RNA)
5 mM AMP-PNP (-RNA)
0 mM AMP-PNP (+RNA)
5 mM AMP-PNP (+RNA)

f

GFP tagged Ddx3xb   Hoechst   Merge

Control

Oligomycin A

g

***

Control   Oligomycin A

h

Endogenous Ddx3xb

Control   Oligomycin A   Oligomycin A + ATP

i

***   ***

Control
Oligomycin A
Oligomycin A + ATP

Supplementary information, Fig. S3   ATP and RNA positively contributes to phase separation of zebrafish Ddx3xb. a, Total ATP levels (in picomoles/embryo) during zebrafish embryogenesis. n = 3 biological replicates per stage. Error bars, mean ± SD. *P* values were determined by the two-tailed Student's t test, **, *P* < 0.01. **b,** A time-course assay monitoring the droplets formation of Alexa-488 labeled Ddx3xb. Scale bars, 10 μm. **c,** The SYTO 64-stained RNA in droplets were associated with fluorescence recovery. Left, representative images of fluorescence recovery. Scale bars, 2 μm. Right, changes in the fluorescence intensity of SYTO 64-stained RNA droplets after photobleaching were plotted over time. The black curve represents the mean of the fluorescence intensity of photobleached regions in different droplets (n=5). Error bars, mean ± SD. **d,** phase separation analysis of Ddx3xb in response to AMP-PNP in the presence of RNA or not. Scale bars, 10 μm. **e,** Quantification of the phase separation analysis as shown in (**d**). n = 20 fields per condition. Error bars, mean ± SD. *P* values were determined by the two-tailed Student's *t* test, ***, *P* < 0.001. **f,** Phase separation behavior of full-length Ddx3xb in different conditions. HEK293 cells in Oligomycin A treatment group were treated with 5 μM Oligomycin A at 12 hours post plasmid transfection. **g,** Quantification of the number of droplets per cell from assay shown in (**f**). n = 25 cells per condition. Error bars, mean ± SD, *P* values were calculated by the two-tailed Student' s *t*-test, ***, *P* < 0.001. **h,** Immunofluorescence staining showing the expression pattern of endogenous Ddx3xb in control, Oligomycin A treatment, and Oligomycin A treatment combined with ATP injection groups. Scale bars, 10 μm. **i,** Quantification of the number of droplets per cell in images from assay shown in (**h**). n = 20 cells per condition. Error bars, mean ± SD. *P* values were determined by the two-tailed Student's *t* test, ***, *P* < 0.001.