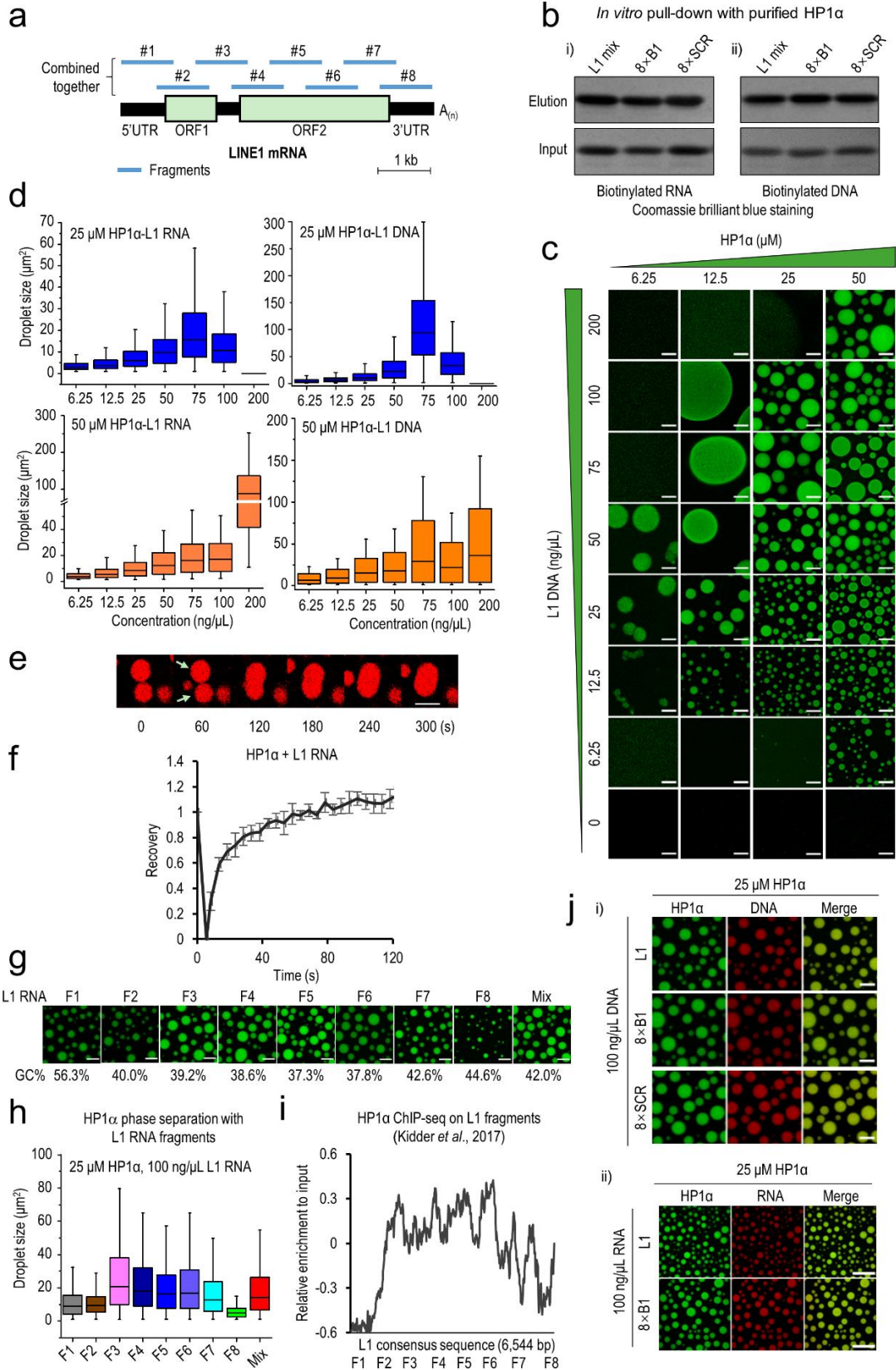


**Fig. S13**



**Fig. S13 HP1 $\alpha$  binds and phase separates with RNA and DNA.**

- (a) Schematic illustrations of L1 fragments used for interaction and phase separation analysis with HP1 $\alpha$ .
- (b) *In vitro* RNA and DNA pull-down of HP1 $\alpha$ .
- (c) Representative images of droplet formation at different concentrations of HP1 $\alpha$  protein and L1 DNA. Concentrations of HP1 $\alpha$  and DNA are indicated at the top and left of the images, respectively. Scale bars, 10  $\mu$ m. Data are representative of 3 independent experiments.
- (d) Boxplot showing the size of droplets formed by HP1 $\alpha$  and L1 RNA/DNA.
- (e) Fluorescence time-lapse microscopy of HP1 $\alpha$ -L1 RNA droplets. Two fusing bodies are indicated by arrows. Scale bars, 10  $\mu$ m. Data are representative of 5 independent experiments.
- (f) FRAP of HP1 $\alpha$ -L1 RNA droplets. The plot shows the time course of the recovery after photobleaching. Time 0 indicates the time of the photobleaching pulse. Scale bar, 10  $\mu$ m. Data are presented as means  $\pm$  SD (N = 6). RNA concentration: 50 ng/ $\mu$ l; HP1 $\alpha$  concentration: 25  $\mu$ M.
- (g) Representative images of droplet formation of HP1 $\alpha$  protein and L1 RNA fragments shown in (a). GC contents of L1 RNA fragment were shown at the bottom.
- (h) Boxplot showing the size of droplets formed by HP1 $\alpha$  and L1 RNA fragments shown in (g).
- (i) HP1 $\alpha$  preferentially binds to the central region of L1 repeat DNA. Relative enrichments of HP1 $\alpha$  ChIP-seq signals normalized to the input (y-axis) are shown across the consensus L1 element (6,544 bp; from F1 to F8), based on HP1 $\alpha$  ChIP-seq in mESCs reported by Kidder et al. (2017)<sup>6</sup>.
- (j) Representative images of droplet formation of HP1 $\alpha$  protein with various DNA (upper: L1, 8xB1 and 8xSCR) and RNA fragments (lower: L1 and 8xB1).

Reference

- 6. Kidder, B. L., Hu, G., Cui, K. & Zhao, K. SMYD5 regulates H4K20me3-marked heterochromatin to safeguard ES cell self-renewal and prevent spurious differentiation. *Epigenetics Chromatin* **10**, 8, doi:10.1186/s13072-017-0115-7 (2017).