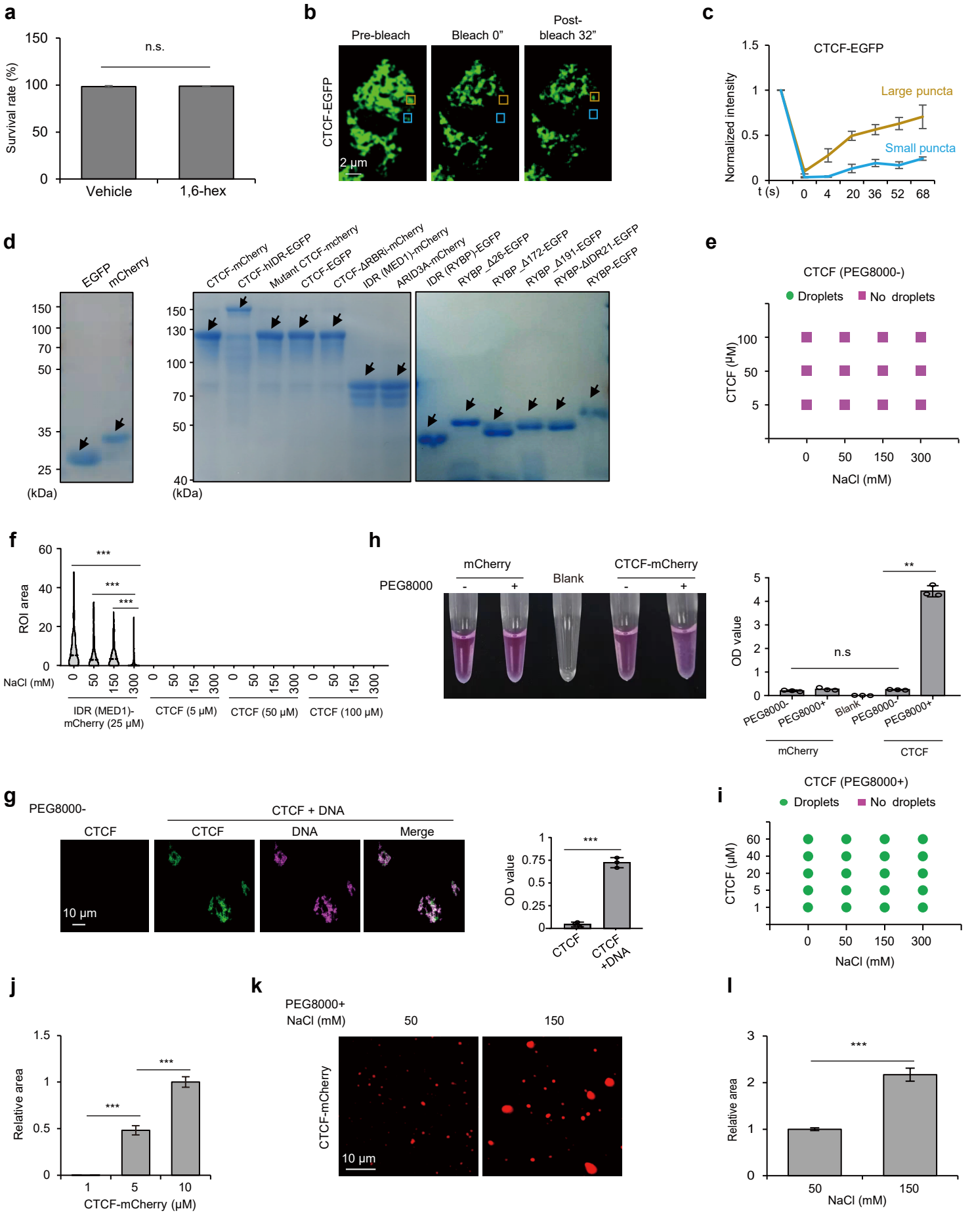
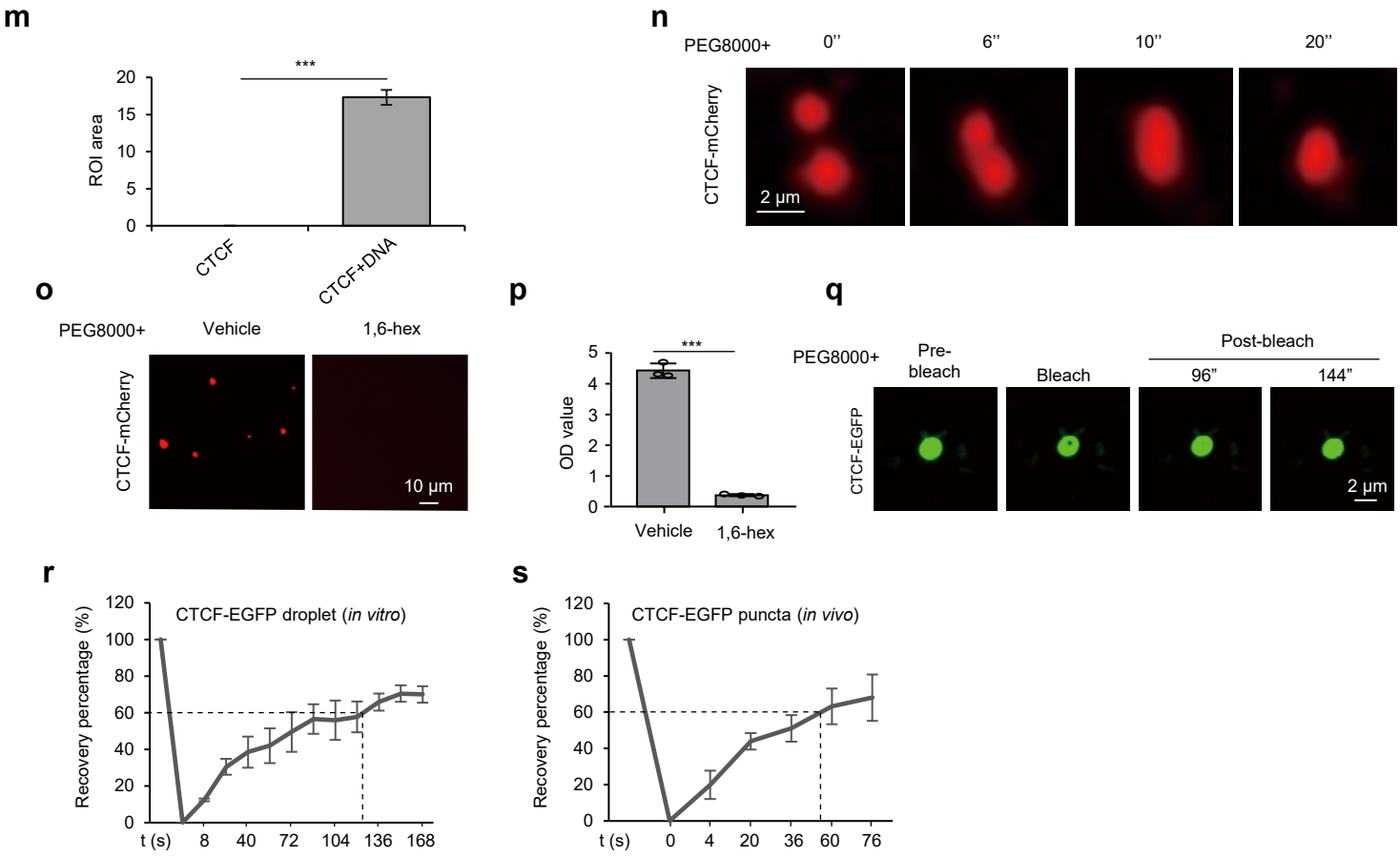
Supplementary information, Fig.S2




m
***
ROI area
20
15
10
5
0
CTCF
CTCF+DNA
n
PEG8000+
0''
6''
10''
20''
CTCF-mCherry
2 μm
o
PEG8000+
Vehicle
1,6-hex
CTCF-mCherry
10 μm
p
***
OD value
5
4
3
2
1
0
Vehicle
1,6-hex
q
PEG8000+
Pre-bleach
Bleach
Post-bleach
96''
144''
CTCF-EGFP
2 μm
r
CTCF-EGFP droplet (in vitro)
Recovery percentage (%)
120
100
80
60
40
20
0
t (s)
8
40
72
104
136
168
s
CTCF-EGFP puncta (in vivo)
Recovery percentage (%)
120
100
80
60
40
20
0
t (s)
0
4
20
36
60
76

Supplementary information, Fig. S2 CTCF exhibits phase separation behavior in the nucleus. a The survival rate of mESCs before and after with1,6-hex treatment. Welch's *t*-test; vehicle, n = 306; 1,6-hex, n = 680; *P* = 0.7235. **b, c** Representative images (**b**) and quantification (**c**) of FRAP in ESCs expressing exogenous CTCF-EGFP. The yellow boxes highlight the bleached large CTCF puncta, and cyan boxes highlight the bleached small CTCF puncta. Data are plotted as means ± SEM (n = 3). **d** SDS-PAGE gel images showed the purity of recombinant proteins, black arrows denote the targeted proteins. **e** Phase diagrams showed CTCF droplet formation under different protein and salt ion concentrations without PEG8000. **f** ROI area of IDR (MED1) and CTCF at different protein and salt ion concentrations without PEG8000. Welch's *t*-test; n values of the IDR (MED1) are (from left to right): 102, 92, 200 and 168, other n values are 0. P values (from left to right): 2.2896e-11, 6.88e-7, 1.391e-13. **g** Without PEG8000, representative images (left) and turbidity assay (right) of CTCF-mCherry aggregation after addition of Cy5-labbled 25×DNA motif (5 ng/μL). The concentration of CTCF-mCherry was 10 μM. Welch's *t*-test; all n values are: n = 3; P =0.0005. **h** Visualization (left) and quantitation (right) of turbidity associated with droplet formation across mCherry and CTCF-mCherry recombinant protein in the presence or absence of PEG8000, the protein concentration of both mCherry and CTCF-mCherry were 60 μM. Welch's *t*-test; all n values are: n = 3; P values (from left to right): 0.1172 and 0.001031. **i** Phase diagrams showed CTCF droplet formation under different protein and salt ion concentrations with PEG8000. **j** Histogram showing the relative ROI area of droplets in the buffer with 1 μM, 5 μM and 10 μM CTCF recombinant protein in the presence of PEG8000. Welch's *t*-test; 1 μM CTCF, n = 37; 5 μM CTCF, n = 162; 10 μM CTCF, n = 1387; P values are (from left to right): p-value < 2.2e-16; *P* = 6.492e-12. **k, l** Representative images (**k**) and histogram (**l**) showed the droplet formation at different NaCl concentrations in the presence of 20% PEG8000, the protein concentration of CTCF-mCherry was 4 μM. Welch's *t*-test; 50 mM NaCl, n

= 997; 150 mM NaCl, n = 762; *P* = 7.284e-16. **m** ROI area of CTCF-mCherry aggregation after addition of Cy5-labbled 25×DNA motif (5 ng/μL). The concentration of CTCF-mCherry was 0.8 μM, 20% PEG8000 were added. Welch's *t*-test; n values are (from left to right): *n* = 0; *n* = 187; *P* < 2.2e-16. **n** Droplet fusion behavior of CTCF-mCherry recombinant protein (4 μM) in the presence of PEG8000. **o, p** Representative images (**o**) and turbidity assay (**p**) of CTCF-mCherry (4 μM) formed droplets before and after treatment with 10% 1,6-hex in the presence of PEG8000, Welch's *t*-test, n = 997, *P* = 0.0009217. **q, r** Representative images (**q**) and quantification (**r**) of FRAP data for CTCF-EGFP droplets *in vitro,* the concentration of CTCF-mCherry was 5 μM. **s** Quantification of FRAP data for CTCF-EGFP puncta *in vivo.*