Expanded View Figures

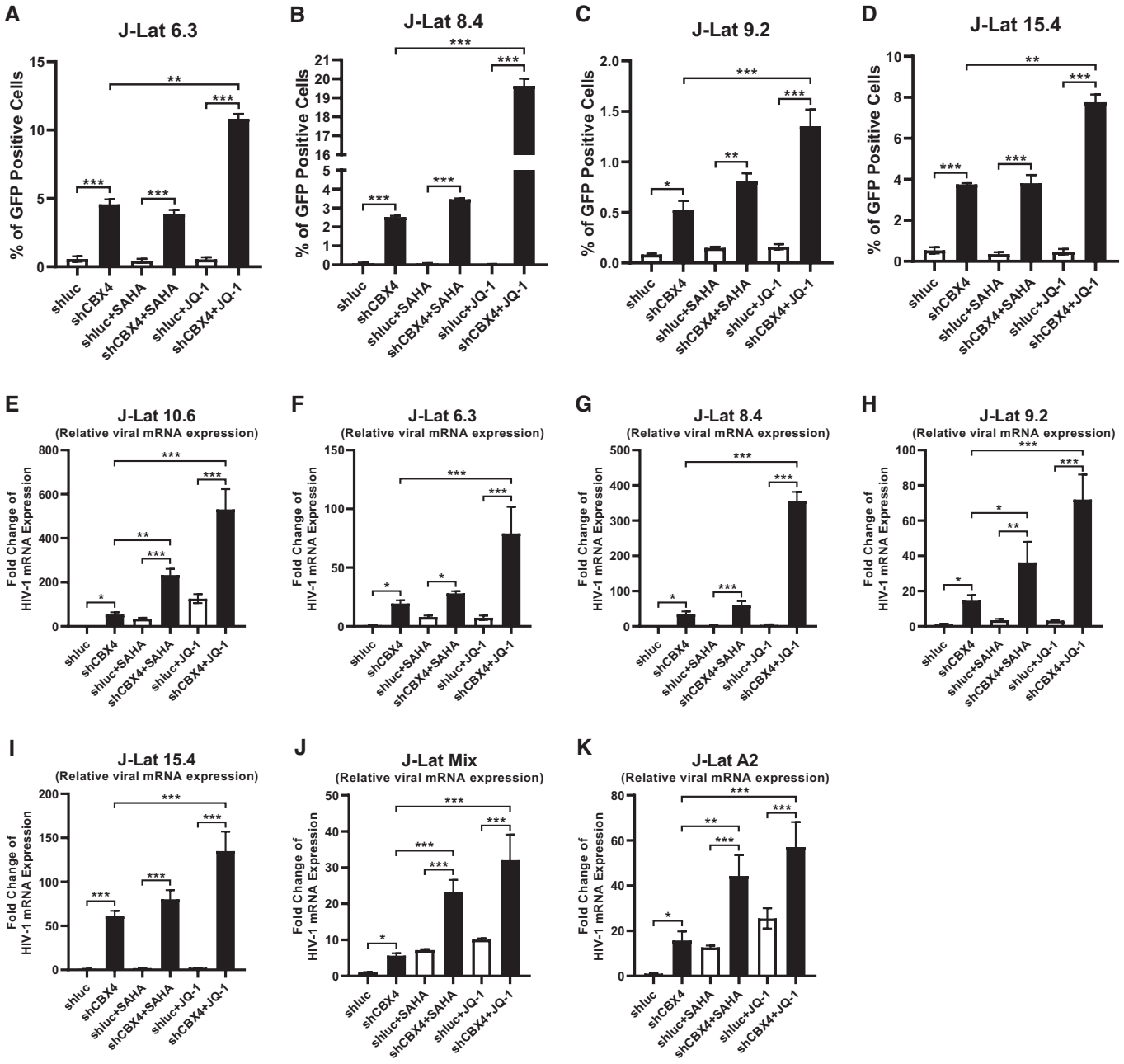

Figure EV1. CBX4 depletion reactivates latent HIV-1.

A–D Monoclonal latency models including J-Lat 6.3, 8.4, 9.2 and 15.4 were treated as in Fig 1F. The reactivation efficiencies of each group are indicated by the percentages of GFP-positive cells.

E–K J-Lat 10.6, 6.3, 8.4, 9.2, 15.4, Mix and A2 were treated as in Fig 1F. Relative HIV-1 mRNA expression within each group was quantified and normalized to the shluc group in each cell line.

Data information: Data are presented as mean ± SEM in biological triplicate. *P*-values were calculated by one-way ANOVA with Tukey's multiple comparisons test.
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Figure EV2. CBX4 bodies colocalize with latent HIV-1 proviruses and EZH2.

A, B ImmunoFISH images of the pseudotyped HIV-1 genomic DNA and CBX4 bodies in J-Lat 10.6 and 8.4. Naïve cells were treated with DMSO. Activated cells were treated with TNFα. The right images show amplified regions.

C, D Distances of the nearest CBX4 body and the HIV-1 provirus in both naïve and activated cells were measured with Imaris software. In each group, 15 cells were randomly imaged and measured the CBX4-HIV-1 distances. The quantification of distances was evaluated in both J-Lat 10.6 (C) and 8.4 (D) cells.

E The endogenous CBX4 and EZH2 in HEK293T cells were captured with rabbit anti-CBX4 antibody and mouse anti-EZH2 antibody respectively. These cells were further incubated with AF488-conjugated anti-rabbit IgG antibody and CF568-conjugated anti-mouse IgG antibody. DAPI indicated DNA. The co-localization was evaluated by line scan profile and shown on the right. The red arrow indicates the profiled position.

F–I GFP-tagged CBX2, CBX6, CBX7 and CBX8 were co-overexpressed with RFP-tagged EZH2. SIM imaging was performed for each combination. Line scan profiles are shown on the right of each panel. Red arrows in merged images indicate the positions where line scans are profiled.

J HA-tagged CBX4 and GFP were co-overexpressed with Flag-tagged EZH2. HA-tagged proteins were immunoprecipitated (IP) with anti-HA beads. In both groups, the IP reactions were supplemented with DNase/RNase or left untreated. Both total and IP samples were immunoblotted (IB) with anti-HA and anti-Flag antibodies.

K ChIP assays with antibodies against IgG and EZH2 were performed in siNC and siCBX4 TZM-bl cells.

Data information: Scale bars in (A), (B) and (E–I) represent 5 μm. Scale bars in amplified images within (A) and (B) represent 500 nm. All the samples were imaged to obtain at least three sets of images. Data in (C) and (D) represent distances in biological replicates ($n = 15$ for C and D). The central bands of the boxplots represent median values of distances. The heights of the boxes represent the interquartile ranges of distances. The boundaries of the upper whiskers and the lower whiskers represent the maximum distances and the minimum distances, respectively. Data in (K) are presented as mean ± SEM in biological triplicate. *P*-values in (C), (D) and (K) were calculated by Student's *t*-test. $^{**}P < 0.01$, $^{***}P < 0.001$.

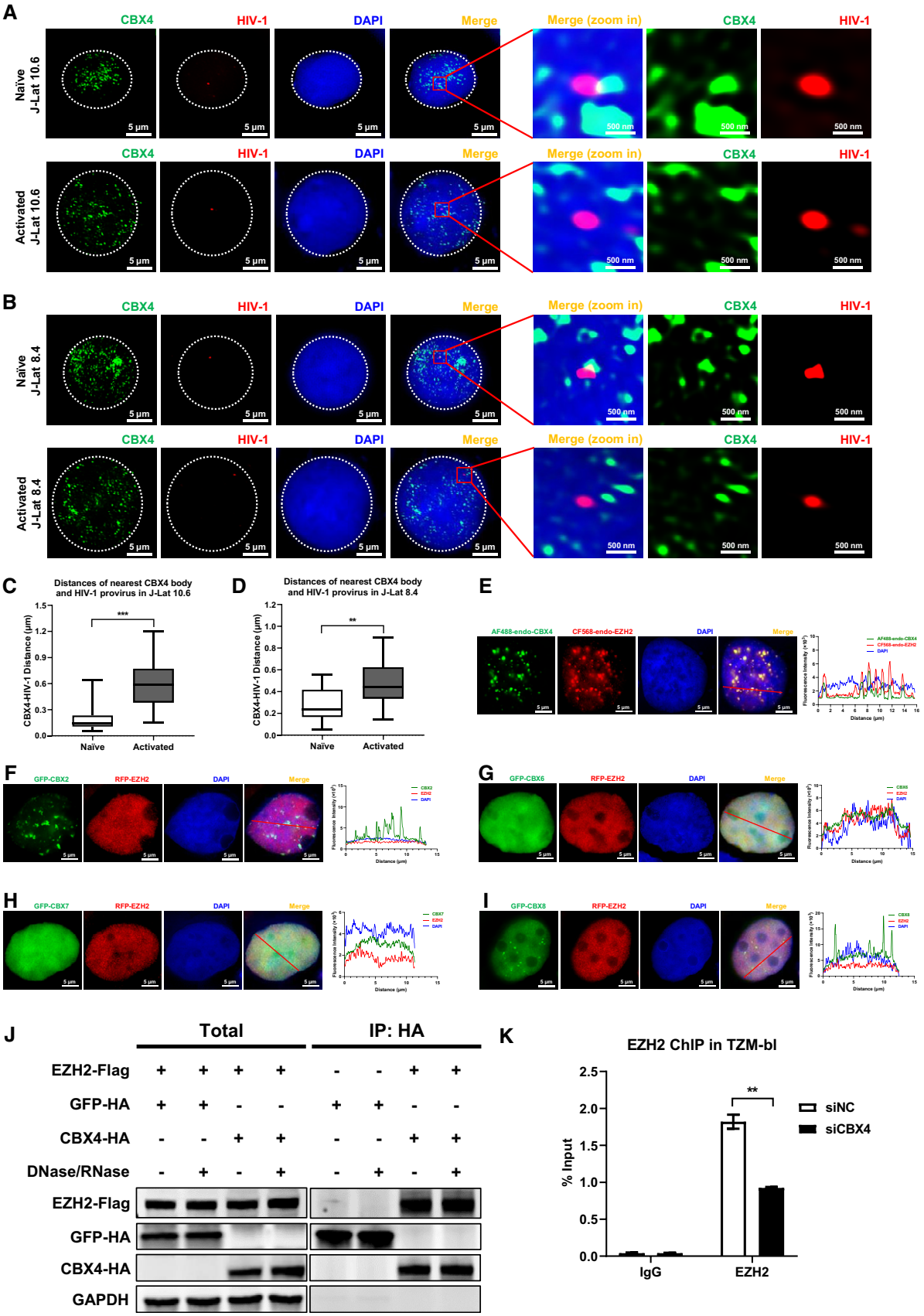
A
CBX4
HIV-1
DAPI
Merge
Merge (zoom in)
Naïve J-Lat 10.6
Activated J-Lat 10.6
5 μm
500 nm
B
Naïve J-Lat 8.4
Activated J-Lat 8.4
C
Distances of nearest CBX4 body and HIV-1 provirus in J-Lat 10.6
CBX4-HIV-1 Distance (μm)
***
Naïve
Activated
D
Distances of nearest CBX4 body and HIV-1 provirus in J-Lat 8.4
**
E
AF488-endo-CBX4
CF568-endo-EZH2
Distance (μm)
F
GFP-CBX2
RFP-EZH2
G
GFP-CBX6
H
GFP-CBX7
I
GFP-CBX8
J
Total
IP: HA
EZH2-Flag
GFP-HA
CBX4-HA
DNase/RNase
GAPDH
K
EZH2 ChIP in TZM-bl
% Input
siNC
siCBX4
IgG
EZH2

Figure EV2.

Figure EV3. Proteins in CBX4 bodies were internally diffused.

A The schematic of CBX4 disorder regions. P1 represents the N-terminal Chromodomain (CD). P2 and P3 represent two sub-regions of IDR. P3 represents C-terminal CBox domain.

B Composition analysis of CBX4 protein. The lower table indicates the distribution of 20 kinds of amino acids.

C–E FRAP images of CBX4 bodies and recruited proteins including EZH2 (C), RING1B (D) and SUMO4 (E). RFP-tagged EZH2, RING1B and SUMO4 were co-overexpressed with GFP-tagged CBX4 in HEK293T cells. Live cell images were captured every 5 s. In each combination, six co-localization bodies were proceeded to FRAP. Three bodies (ROI 1, ROI 2 and ROI 3) were left untreated. Another three bodies (ROI 4, ROI 5 and ROI 6) were bleached with strong 488 and 561 nm laser power. The right histograms show relative fluorescence intensities of unbleached and bleached CBX4 and its partners in each time point.

F The numbers of CBX4 bodies before 1,6-Hex treatment (0 min) and after 1,6-Hex treatment (2 min) were calculated.

G Cellular viabilities before 1,6-Hex treatment (0 min) and after 1,6-Hex treatment (2 min) were evaluated by measuring the percentages of amine-reactive fluorescent dye non-permeant cells.

Data information: Scale bars in (C–E) represent 5 μm. All the samples were imaged to obtain at least three sets of images. Data are presented as mean ± SEM in biological triplicate. *P*-values in (F) and (G) were calculated by Student's *t*-test. $^{**}P < 0.01$.

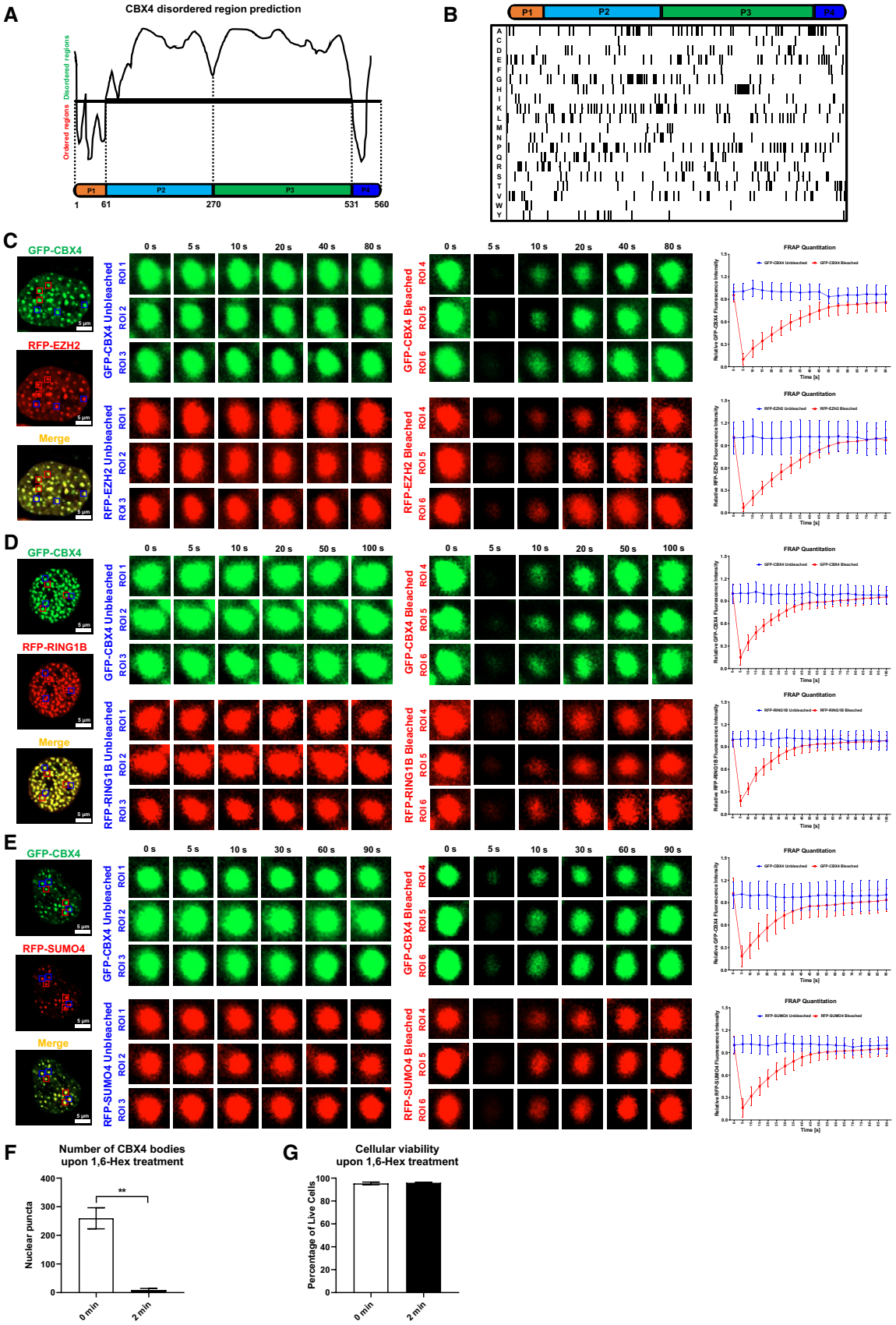
A
CBX4 disordered region prediction
Disordered regions
Ordered regions
P1
P2
P3
P4
1
61
270
531
560
B
C
GFP-CBX4
RFP-EZH2
Merge
GFP-CBX4 Unbleached
GFP-CBX4 Bleached
RFP-EZH2 Unbleached
RFP-EZH2 Bleached
0 s
5 s
10 s
20 s
40 s
80 s
FRAP Quantitation
D
RFP-RING1B
RFP-RING1B Unbleached
RFP-RING1B Bleached
50 s
100 s
E
RFP-SUMO4
RFP-SUMO4 Unbleached
RFP-SUMO4 Bleached
30 s
60 s
90 s
F
Number of CBX4 bodies upon 1,6-Hex treatment
Nuclear puncta
0 min
2 min
G
Cellular viability upon 1,6-Hex treatment
Percentage of Live Cells

Figure EV3.

Figure EV4. The LLPS of CBX4 depends on the β-sheet motifs of CBox.

A About 25 μM of *in vitro* purified GFP-CBX4 proteins were incubated with gradient droplet formation buffers. GFP-CBX4 droplets appeared in 250, 125, 62.5, 31.25 and 15.625 mM NaCl. The right subpanel represents statistical analysis of droplet areas and numbers of GFP-CBX4 within different NaCl concentrations.

B Different concentrations of *in vitro* purified GFP-CBX4 proteins were incubated with 75 mM NaCl droplet formation buffer. The right subpanel represents statistical analysis of droplets areas and numbers of GFP-CBX4 within different protein concentrations.

C, D The *in vitro* purified GFP-CBX4mut proteins (25 μM) were incubated with different droplet formation buffers which contained different NaCl concentrations (C). Different concentrations of GFP-CBX4mut proteins were incubated with 75 mM NaCl droplet formation buffer (D). The right subpanel within each panel represents the statistical analysis of droplets areas and numbers of GFP-CBX4mut within different conditions.

E The purities of *in vitro* purified GFP-CBX4 and GFP-CBX4mut proteins were analyzed by both Coomassie blue staining and Western blotting against GFP.

F HA-tagged CBX4 and CBX4mut were co-overexpressed with Flag-tagged EZH2 respectively. HA-tagged proteins were IP with anti-HA beads. Both total and IP samples were IB with anti-HA and anti-Flag antibodies.

G TZM-bl cells were treated with siCBX4 targeting 3'UTR of *CBX4* mRNA or siNC. Another two groups of siCBX4-treated cells were re-overexpressed with wild-type CBX4 and LLPS-deficient CBX4mut respectively. ChIP assays with antibodies against IgG and EZH2 were performed in these cells.

Data information: Scale bars in (A–D) represent 10 μm. All the samples were imaged to obtain at least three sets of images. Data in (G) are presented as mean ± SEM in biological triplicate. *P*-values in (G) were calculated by two-way ANOVA with Tukey's multiple comparisons test. ***$P < 0.001$.

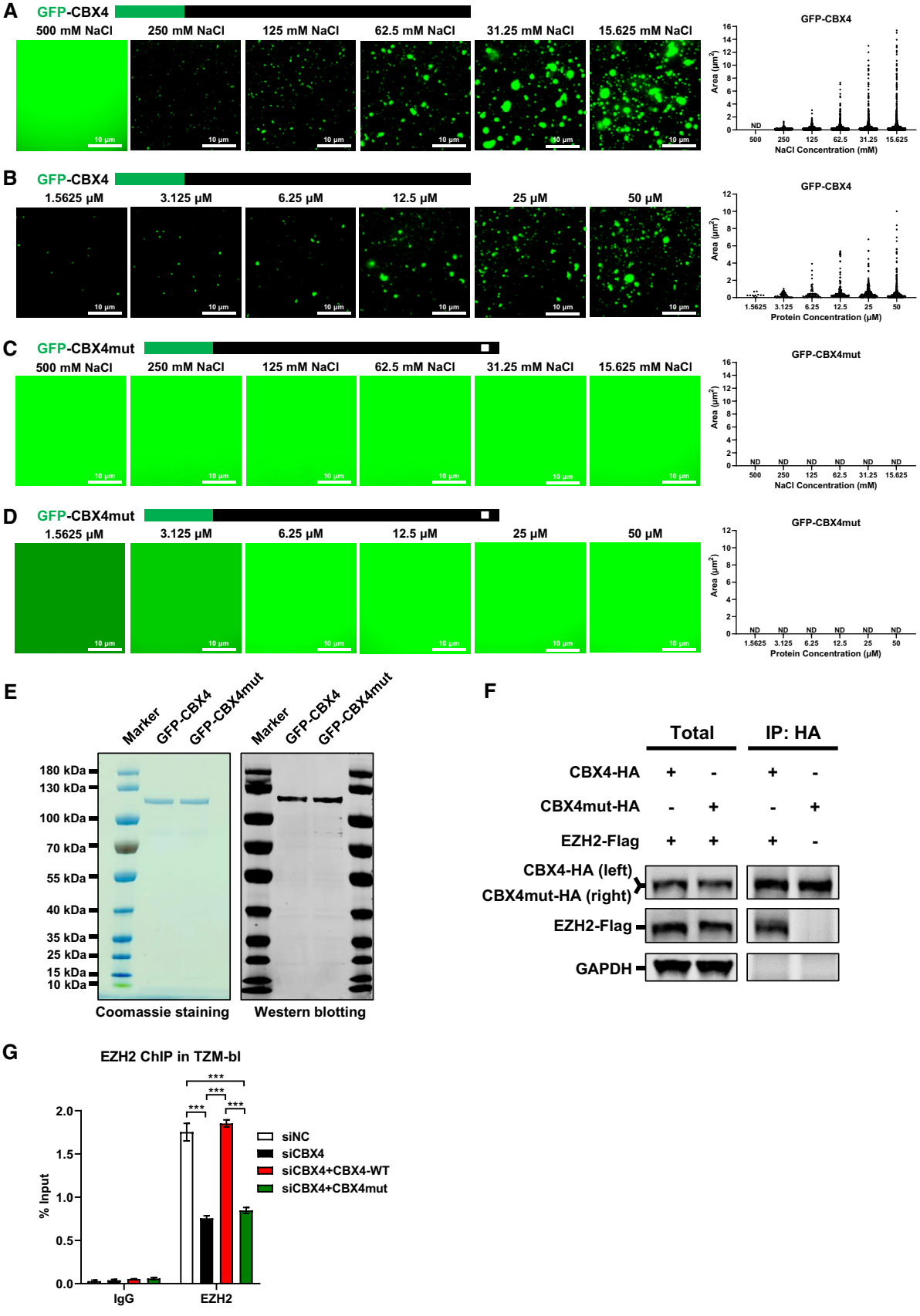
A
GFP-CBX4
500 mM NaCl
250 mM NaCl
125 mM NaCl
62.5 mM NaCl
31.25 mM NaCl
15.625 mM NaCl
GFP-CBX4
Area (μm²)
NaCl Concentration (mM)
B
GFP-CBX4
1.5625 μM
3.125 μM
6.25 μM
12.5 μM
25 μM
50 μM
GFP-CBX4
Protein Concentration (μM)
C
GFP-CBX4mut
500 mM NaCl
250 mM NaCl
125 mM NaCl
62.5 mM NaCl
31.25 mM NaCl
15.625 mM NaCl
GFP-CBX4mut
NaCl Concentration (mM)
D
GFP-CBX4mut
1.5625 μM
3.125 μM
6.25 μM
12.5 μM
25 μM
50 μM
GFP-CBX4mut
Protein Concentration (μM)
E
Marker
GFP-CBX4
GFP-CBX4mut
180 kDa
130 kDa
100 kDa
70 kDa
55 kDa
40 kDa
35 kDa
25 kDa
15 kDa
10 kDa
Coomassie staining
Western blotting
F
Total
IP: HA
CBX4-HA
CBX4mut-HA
EZH2-Flag
CBX4-HA (left)
CBX4mut-HA (right)
EZH2-Flag
GAPDH
G
EZH2 ChIP in TZM-bl
% Input
siNC
siCBX4
siCBX4+CBX4-WT
siCBX4+CBX4mut
IgG
EZH2

Figure EV4.

Figure EV5. CBX4 SUMOylates EZH2 with SUMO1, SUMO2 and SUMO4.

A HA-tagged EZH2 was co-overexpressed with Flag-tagged SUMO1, GFP-tagged UBC9 or Flag-tagged CBX4. EZH2 was IP with anti-HA beads. Both total and IP samples were IB with anti-HA, -Flag, and -GAPDH antibodies. The expression ratios of SUMO1-EZH2 are marked below the panel.

B Target proteins were overexpressed, IP and IB as in (A), except that Flag-tagged SUMO molecule was changed with SUMO2. The expression ratios of SUMO2-EZH2 are marked below the panel.

C Target proteins were overexpressed, IP and IB as in (A), except that Flag-tagged SUMO molecule was changed with SUMO4. The expression ratios of SUMO4-EZH2 are marked below the panel.

D Primary $CD4^+$ T cells from three healthy donors were transfected with siNC or siCBX4 utilizing 4D-Nucleofector System. About 24 h post transfection, the endogenous EZH2 within each group was IP with anti-EZH2 antibodies. Both total and IP samples were IB with anti-EZH2, anti-CBX4 and anti-GAPDH antibodies. The expression ratios of SUMO-EZH2 within each group are marked below the panel.

E HA-tagged EZH2 and EZH2 truncation mutants were co-overexpressed with Flag-tagged SUMO4, Flag-tagged UBC9 and Flag-tagged CBX4. EZH2 and corresponding mutants were IP with anti-HA beads. Both total and IP samples were IB with antibodies against HA, Flag and GAPDH. The asterisks represent SUMOylated EZH2 mutants.

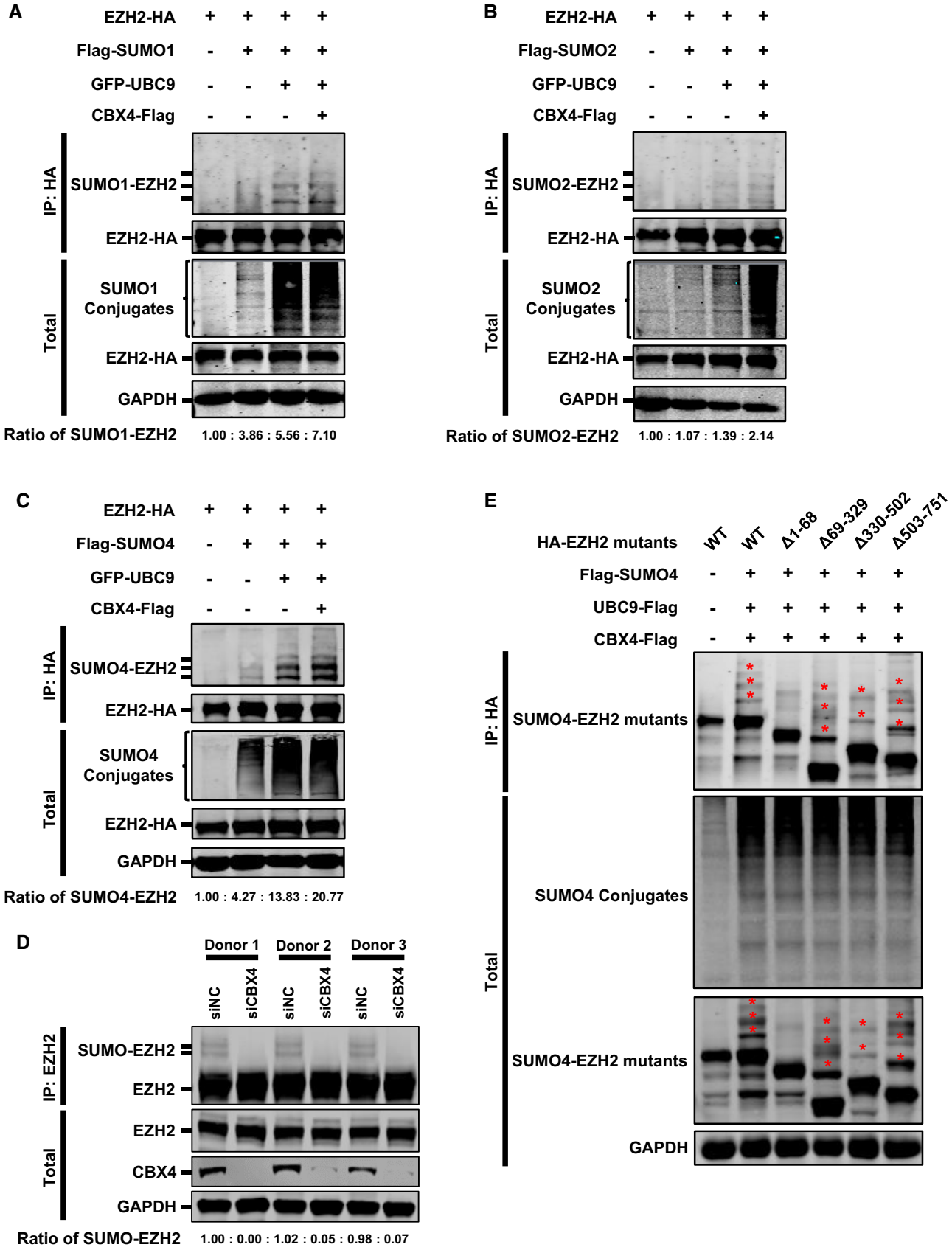
A
EZH2-HA + + + +
Flag-SUMO1 - + + +
GFP-UBC9 - - + +
CBX4-Flag - - - +
IP: HA
SUMO1-EZH2
EZH2-HA
Total
SUMO1 Conjugates
EZH2-HA
GAPDH
Ratio of SUMO1-EZH2 1.00 : 3.86 : 5.56 : 7.10
B
EZH2-HA + + + +
Flag-SUMO2 - + + +
GFP-UBC9 - - + +
CBX4-Flag - - - +
IP: HA
SUMO2-EZH2
EZH2-HA
Total
SUMO2 Conjugates
EZH2-HA
GAPDH
Ratio of SUMO2-EZH2 1.00 : 1.07 : 1.39 : 2.14
C
EZH2-HA + + + +
Flag-SUMO4 - + + +
GFP-UBC9 - - + +
CBX4-Flag - - - +
IP: HA
SUMO4-EZH2
EZH2-HA
Total
SUMO4 Conjugates
EZH2-HA
GAPDH
Ratio of SUMO4-EZH2 1.00 : 4.27 : 13.83 : 20.77
D
Donor 1
Donor 2
Donor 3
siNC
siCBX4
siNC
siCBX4
siNC
siCBX4
IP: EZH2
SUMO-EZH2
EZH2
Total
EZH2
CBX4
GAPDH
Ratio of SUMO-EZH2 1.00 : 0.00 : 1.02 : 0.05 : 0.98 : 0.07
E
HA-EZH2 mutants WT WT Δ1-68 Δ69-329 Δ330-502 Δ503-751
Flag-SUMO4 - + + + + +
UBC9-Flag - + + + + +
CBX4-Flag - + + + + +
IP: HA
SUMO4-EZH2 mutants
Total
SUMO4 Conjugates
SUMO4-EZH2 mutants
GAPDH

Figure EV5.