Monitoring histone methylation (H3K9me3) changes in live cells

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Supporting Methods

Recombinant protein sensors production

Recombinant protein sensor were produced following our previous protocols.[1] Briefly, *E. coli* cells ((DE3)-RIPL, Stratagene, CA, US) were transformed with the pET-21b(+) vector in containing the selected protein sensor in which a flexible linker and His-tag was added in the C-terminus. Protein expression was induced with IPTG at 0.5 mM for 6 h at 37 °C. The protein was then purified using Nickel agarose beads (Qiagen, Hilden, Germany), concentrated and stored in a storage buffer (Tris-HCl, 25 mM, pH 7.2; NaH_2PO_4, 25 mM, pH 7.2; NaCl, 25 mM; EDTA, 1 mM; DTT, 1 mM; and glycerol, 5 % (v/v)) at 4 °C. The quality of the protein was verified using SDS-PAGE and Western blot.

Production of nuclear cell extracts and immuno-blotting assays

Human embryonic kidney 293T (HEK293T) cells were detached from the surface of a culture dish using a solution of 0.05% Trypsin-EDTA. Harvested cells were then lysed to obtain nuclear extracts via a Nuclear Extraction Kit (Abcam, CA, US). Nuclear extracts (NE) were analyzed by dot blot using an anti-histone H3 antibody (ab1791, Abcam, CA, US) and anti-H3K9me3 antibody (ab8898, Abcam, CA, US) as shown in **Fig. S9 (Supporting Information).**

Affinity measurement using Bio-layer Interferometry

Bio-layer interferometry (BLI, OctetRed 384, ForteBio, Menlo Park, CA) was used to assess the affinity and specificity of the selected protein sensor. The non-fluorescence labeled protein sensor was expressed in bacteria and purified as described above. The anti-penta-His biosensor tips (ForteBio, Menlo Park, CA) was used for immobilizing the expressed probes. Wild-type histone H3 proteins were prepared as we described in our previous work.[2] H3K9me3 proteins

were prepared using a methyl-lysine-analogue approach following the established protocol.[3] The binding assay was performed in a pH-controlled buffer (25 mM Tris-HCl, 25 mM, pH 7.2; NaH_2PO_4, 25 mM, pH 7.2; NaCl, 25 mM; EDTA, 1 mM; DTT, 1 mM; BSA, 0.1% w/v; and Tween 20, 0.05%v/v). Similarly, biotinylated H3 histone peptides (with sequence detailed in Table S2) were used to assess the selectivity of the protein sensor towards H3K9me3 and H3K9me2. The synthetic peptides were loaded on streptavidin-activated capture biosensors (ForteBio, Menlo Park, CA). Using the same pH-controlled buffer as before, sensors of concentrations ranging from 0.3 to 2.4 μM were used to determine the binding affinity. The binding and dissociation curves were fitted using Octet software to determine the the k_{on} and k_{off}, which subsequently can be used to determine K_d as k_{off}/k_{on}. Data analysis was performed as we described in our previous work.[4]

Fixed cells antibody or proteins sensor immunostaining

HEK293T cells were seeded on poly-L-Lysine treated coverslips (No. 1.5 round coverslips, VWR, PA, US) and grown for 24 hours. Cells were then fixed using freshly prepared 4% paraformaldehyde in PBS for 20 minutes at room temperature, followed by 10 minutes of permeabilization with 0.2% Triton X-100 in PBS. For antibody immunostaining, coverslips with fixed cells were then incubated with a primary antibody, anti H3K9me3 (ab8898, Abcam, CA, US) at 4˚C overnight. The cells were triple rinsed with PBS, followed by 1 hour of incubation at room temperature with the secondary antibody (an Alexa 564 coupled goat anti rabbit (ab175471, Abcam, CA, US)). A control well was prepared by incubating cells with the secondary antibody in absence of the primary to assess the non-specific activity of the secondary antibody. For protein sensor immunostaining, coverslips with fixed cells were then incubated with the fluorescent labeled protein sensor, ΔCSD- ΔCSD-mEGFP, at 4˚C overnight. The cells were then triple rinsed with PBS.

Table S1. Sequences of synthetic H3 peptides.

Peptide	Amino acid sequence
H3WT	ARTKQTARKSTGGKAPRKQLA-GGK(Biotin)
H3K9me2	ARTKQTAR-K(me2)-STGGKAPRKQLA-GGK(Biotin)
H3K9me3	ARTKQTAR-K(me3)-STGGKAPRKQLA-GGK(Biotin)

Table S2. Detailed amino acid sequences of various sensor designs. H3K9me3 recognition domains are in bold. The SV40 nuclear localization signal (NLS) is double underlined. The linker connecting different domains is underlined. 6×His-tag is included in the c-terminus for purification and validation purpose.

Name	Amino acid sequence
ΔCD	10 20 30 40 50 MPKKKRKVGS GSGSGSGGSE **FYAVEKIIDR RVRKGKVEYY LKWKGYPETE** 60 70 80 90 100 **NTWEPENNLD CQDLIQQYEA SRKDEEKSAA** GSGGGGGSQL GSGGVDSCLP 110 SCLPVRHHHH HH
ΔCD-ΔCD	10 20 30 40 50 MPKKKRKVGS GSGSGSGGSE **FYAVEKIIDR RVRKGKVEYY LKWKGYPETE** 60 70 80 90 100 **NTWEPENNLD CQDLIQQYEA SRKDEEKSAA** GSGGGGGSQ**F YAVEKIIDRR** 110 120 130 140 150 **VRKGKVEYYL KWKGYPETEN TWEPENNLDC QDLIQQYEAS RKDEEKSAA**G 160 170 SGGGGGSQLG SGGVDSCLPV RHHHHHH
ΔCSD	10 20 30 40 50 MPKKKRKVGS GSGSGSGGSE **FLEAEKILGA SDNNGRLTFL IQFKGVDQAE** 60 70 80 90 100 **MVPSSVANEK IPRMVIHFYE ERLSWYSDNE D**GSGGGGGSQ LGSGGVDSCL PVRHHHHHH
ΔCSD- ΔCSD	10 20 30 40 50 MPKKKRKVGS GSGSGSGGSE **FLEAEKILGA SDNNGRLTFL IQFKGVDQAE** 60 70 80 90 100 **MVPSSVANEK IPRMVIHFYE ERLSWYSDNE D**GSGGGGGSQ **FLEAEKILGA** 110 120 130 140 150 **SDNNGRLTFL IQFKGVDQAE MVPSSVANEK IPRMVIHFYE ERLSWYSDNE** 160 170 **D**GSGGGGGSQ LGSGGVDSCL PVRHHHHHH
ΔCD - ΔCSD	10 20 30 40 50 MPKKKRKVGS GSGSGSGGSE **FYAVEKIIDR RVRKGKVEYY LKWKGYPETE** 60 70 80 90 100 **NTWEPENNLD CQDLIQQYEA SRKDEEKSAA** GSGGGGGSQ**F LEAEKILGAS** 110 120 130 140 150 **DNNGRLTFLI QFKGVDQAEM VPSSVANEKI PRMVIHFYEE RLSWYSDNED** 160 170 GSGGGGGSQL GSGGVDSCLP VRHHHHHH

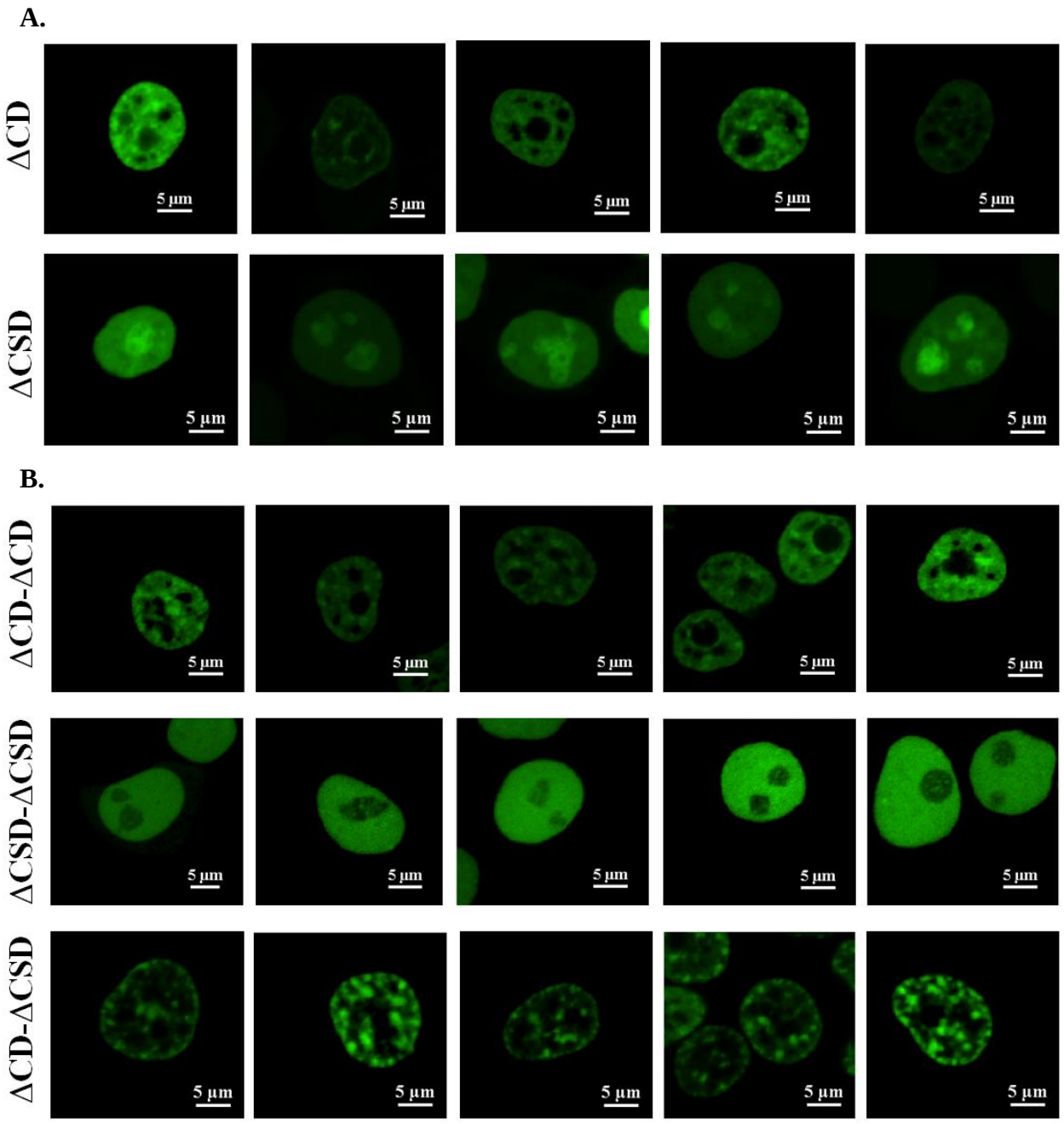
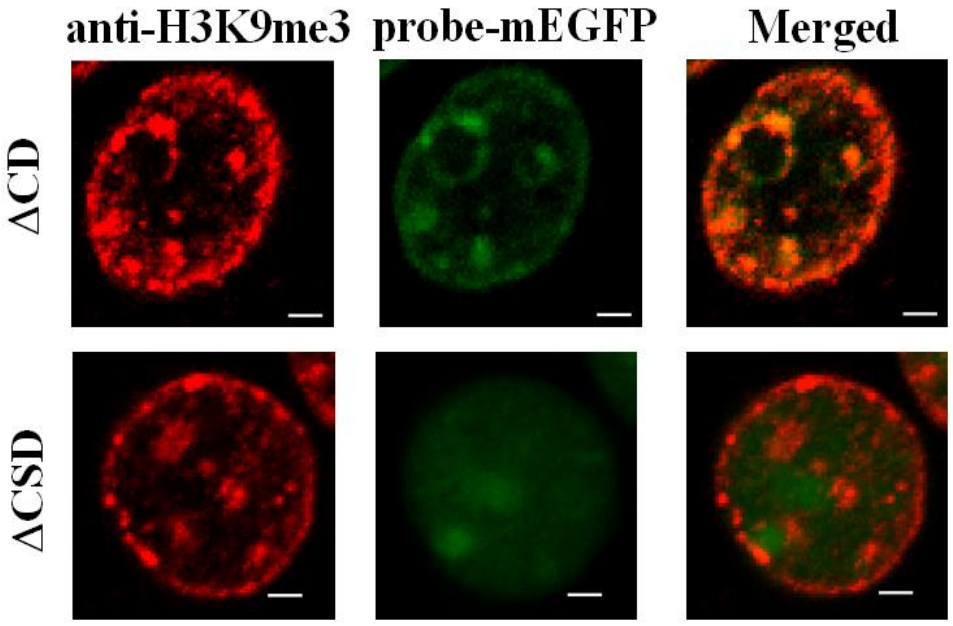
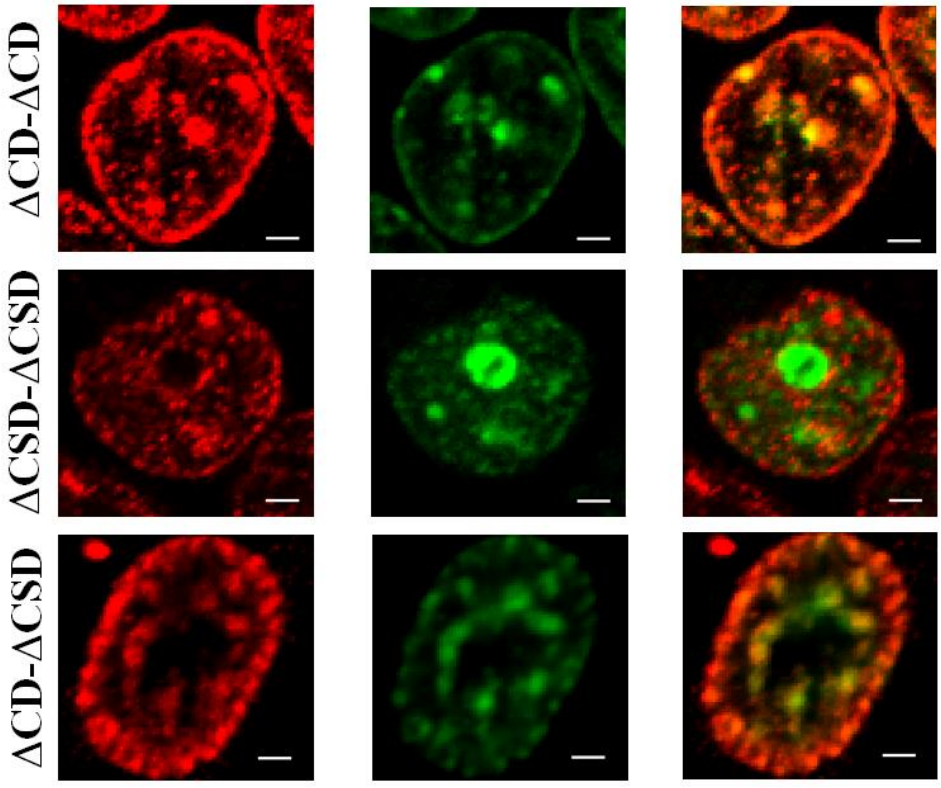


Figure S1. Representative images of HEK293T cells transfected with **A**. monomeric and **B.** dimeric chromodomain protein sensors. All cell images are 2D slices.

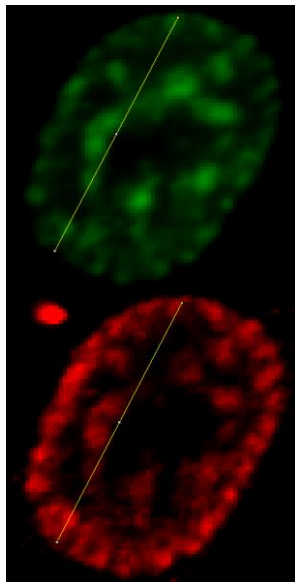
A.

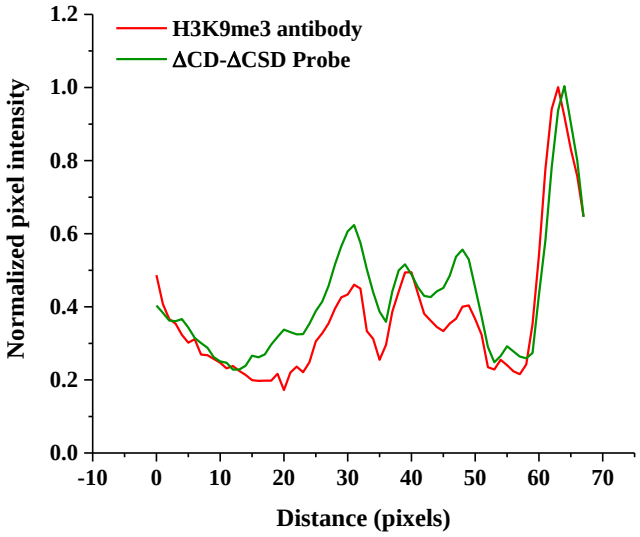

anti-H3K9me3
probe-mEGFP
Merged
ΔCD
ΔCSD

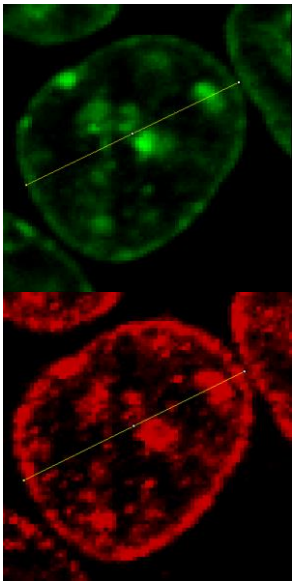
B.


ΔCD-ΔCD
ΔCSD-ΔCSD
ΔCD-ΔCSD

C.







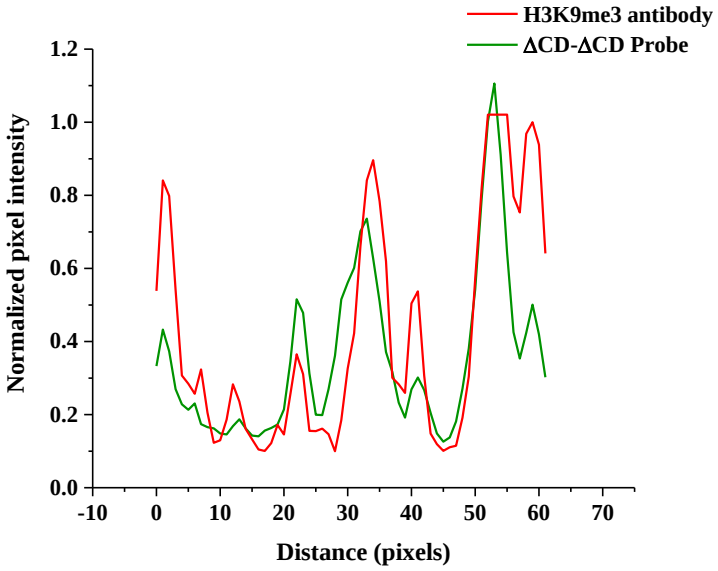


Figure S2. Representative images of transfected HEK293T cells with **A.** monomeric and **B.** dimeric sensors and co-stained with anti-H3K9me3 antibody and an Alexa 564 secondary antibody. **C.** Intensity profile for the cells transfected with sensor ΔCD-ΔCSD and ΔCD-ΔCD.

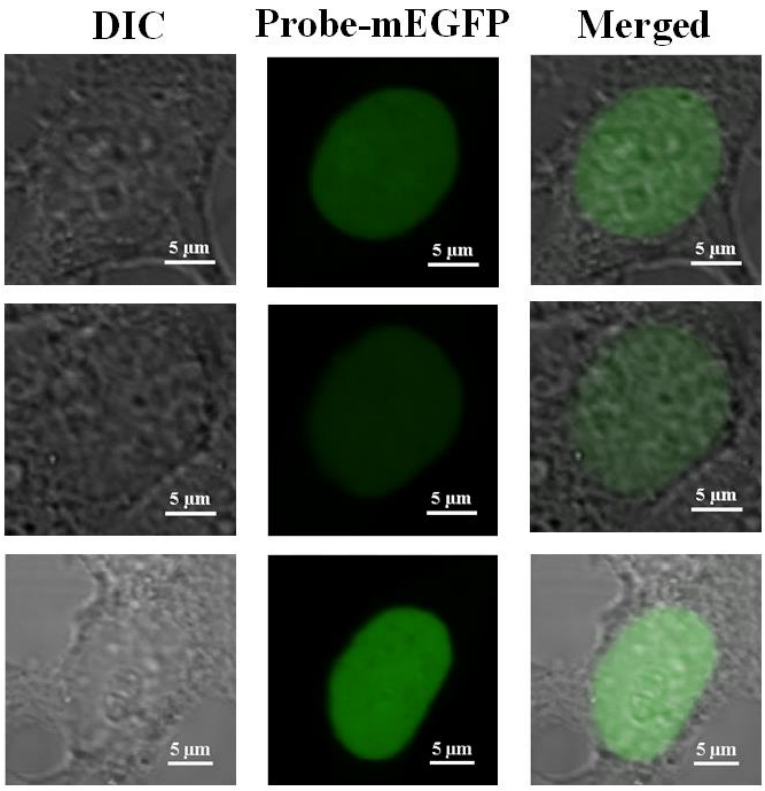


Figure S3. Representative images of HEK293T cells transfected with the mutated protein sensor ΔCD(KW42/43AA)-ΔCSD

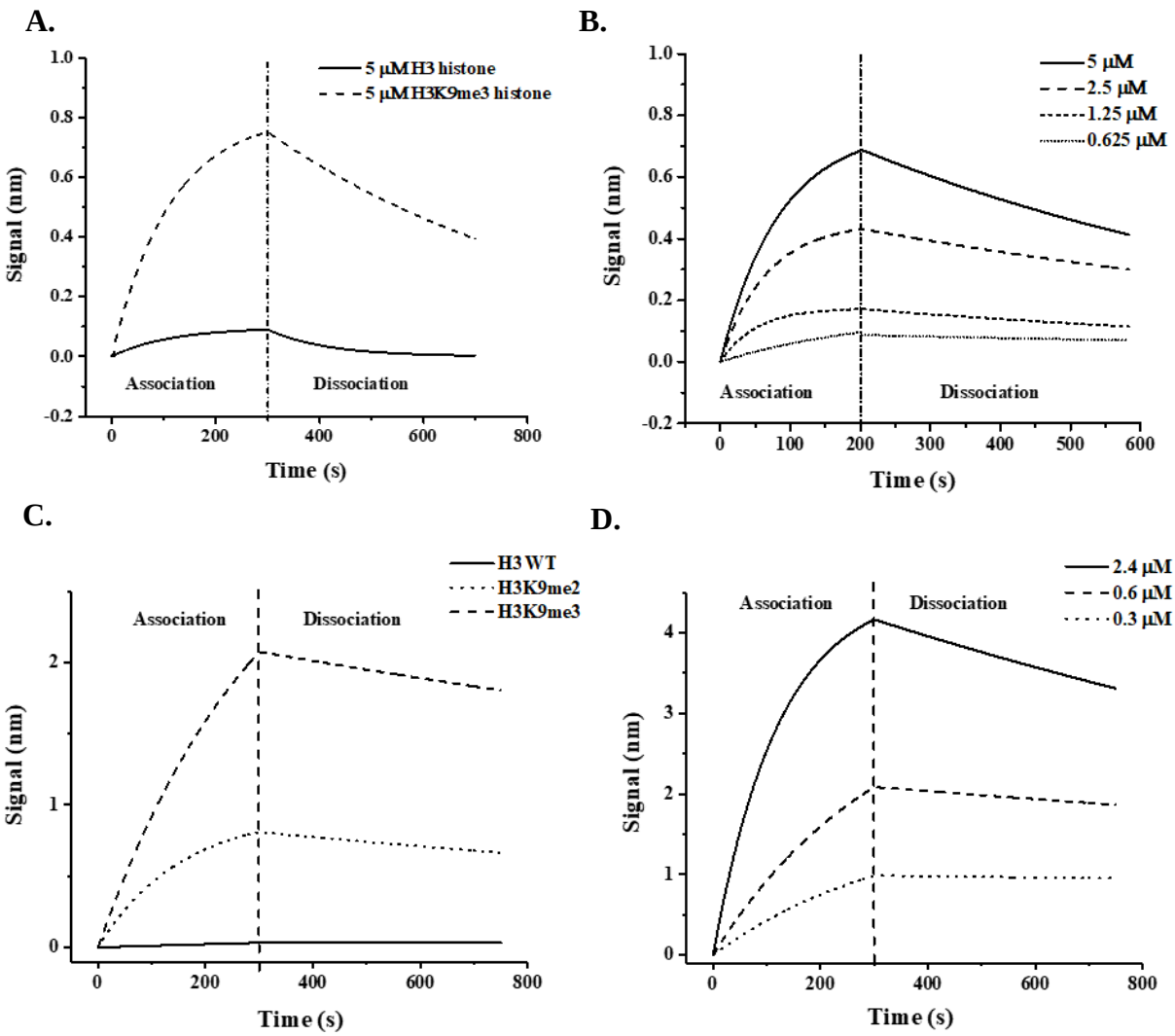


Figure S4. Affinity measurements via bio-layer interferometry (BLI). **A.** Comparison of binding curves of ΔCD-ΔCSD with wild-type H3 and H3K9me3 protein. [Protein] = 5 μM. Interaction with H3K9me3 histone exhibited a discernible association and dissociation curve, which can subsequently be used to determine the binding affinity. **B.** Binding affinity of the sensor and H3K9me3 proteins was evaluated by varying the protein concentrations from 0.62 to 5 μM. A global fitting was then performed to determine the value of K_D. K_D was found to be 0.24 ± 0.02 μM as plotted. **C.** Comparison of binding curves of ΔCD-ΔCSD with biotinylated synthetic H3 peptides. [Protein] = 0.6 μM. Interaction with H3K9me3 peptide is larger compared to H3K9me2 peptide, no probe:H3 wild type interaction is observed. **D.** Binding affinity of the sensor and H3K9me3 peptide was evaluated by varying the protein concentrations from 0.3 to 2.4 μM. K_D was found to be 0.54 ± 0.16 μM as plotted.

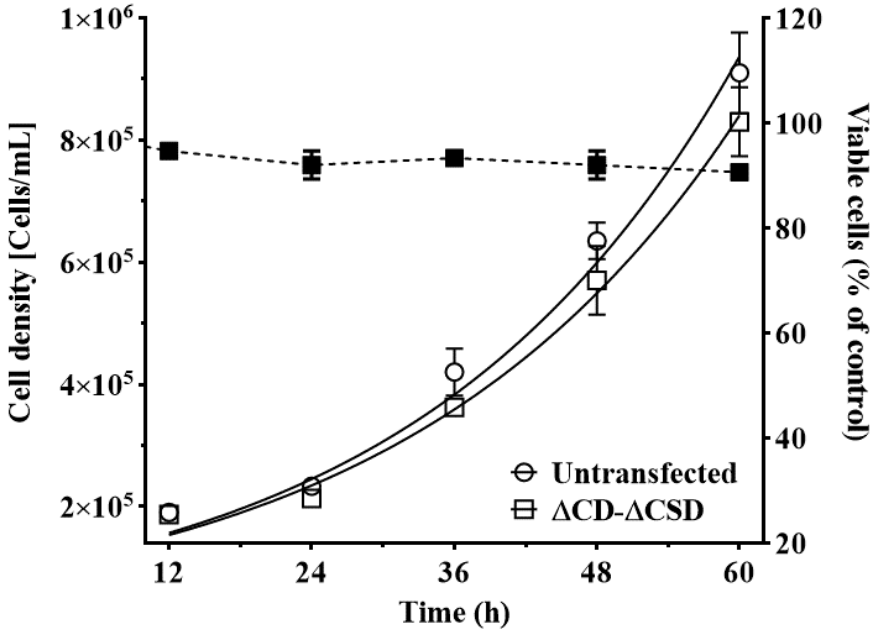


Figure S5. Growth curve (○, untransfected and □, transfected) and viability (■: transfected relative to untransfected) of cells. Neither the growth or viability of transfected cells were significantly altered ($p > 0.05$) compared with the untransfected control during our observation window.

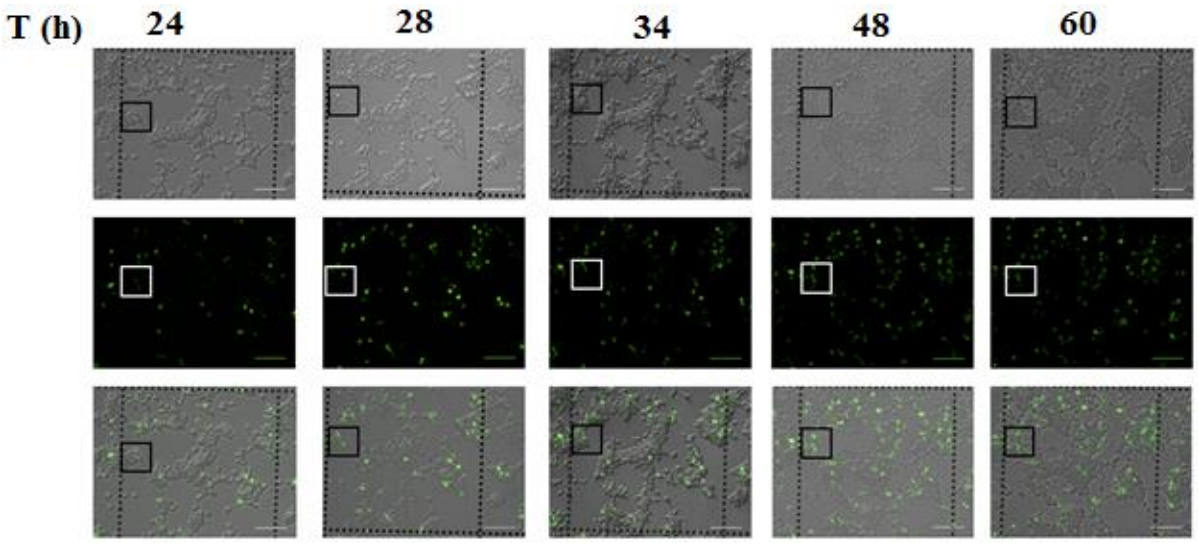
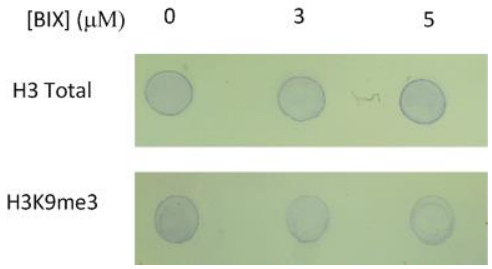


Figure S6. Selection of a defined area using cells transfected with the ΔCD-ΔCSD protein sensor over successive time points. A gridded slide was used to monitor the same area. The area was imaged using a 10× objective to provide a view of the entire box. T indicates the time in hours after transfection and the scale bars = 100 μM. Top, middle and bottom rows present the same area in DIC, probe-GFP and merged channels, respectively. The square in different images indicates the area that has been zoom in in **Fig. 3**.

A.



B.

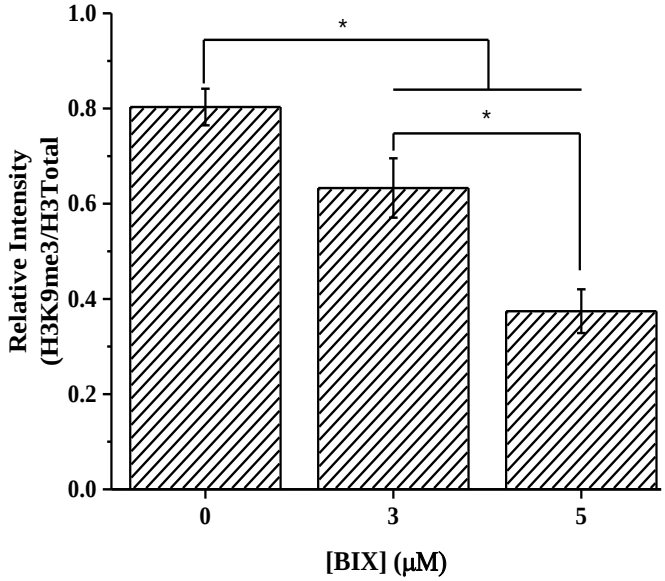


Figure S7. Effect of BIX-01294 on global H3K9me3 of HEK293T cells after 48 h of exposure. **A.** Representative dot blot images of nuclear extracts. Detection of histone H3 and H3K9me3 was conducted using the primary antibodies ab1791 and ab8898 (Abcam, US), respectively, and a goat anti-rabit IgG antibody HRP-conjugated (12-348, Millipore, US) as a secondary. **B.** H3K9me3 levels were expected to be proportional to the relative intensity of H3K9me to total H3 quantified from dot blot samples. Data is presented as mean of three independent assays. *: $p < 0.05$; denotes statistical difference among compared groups.

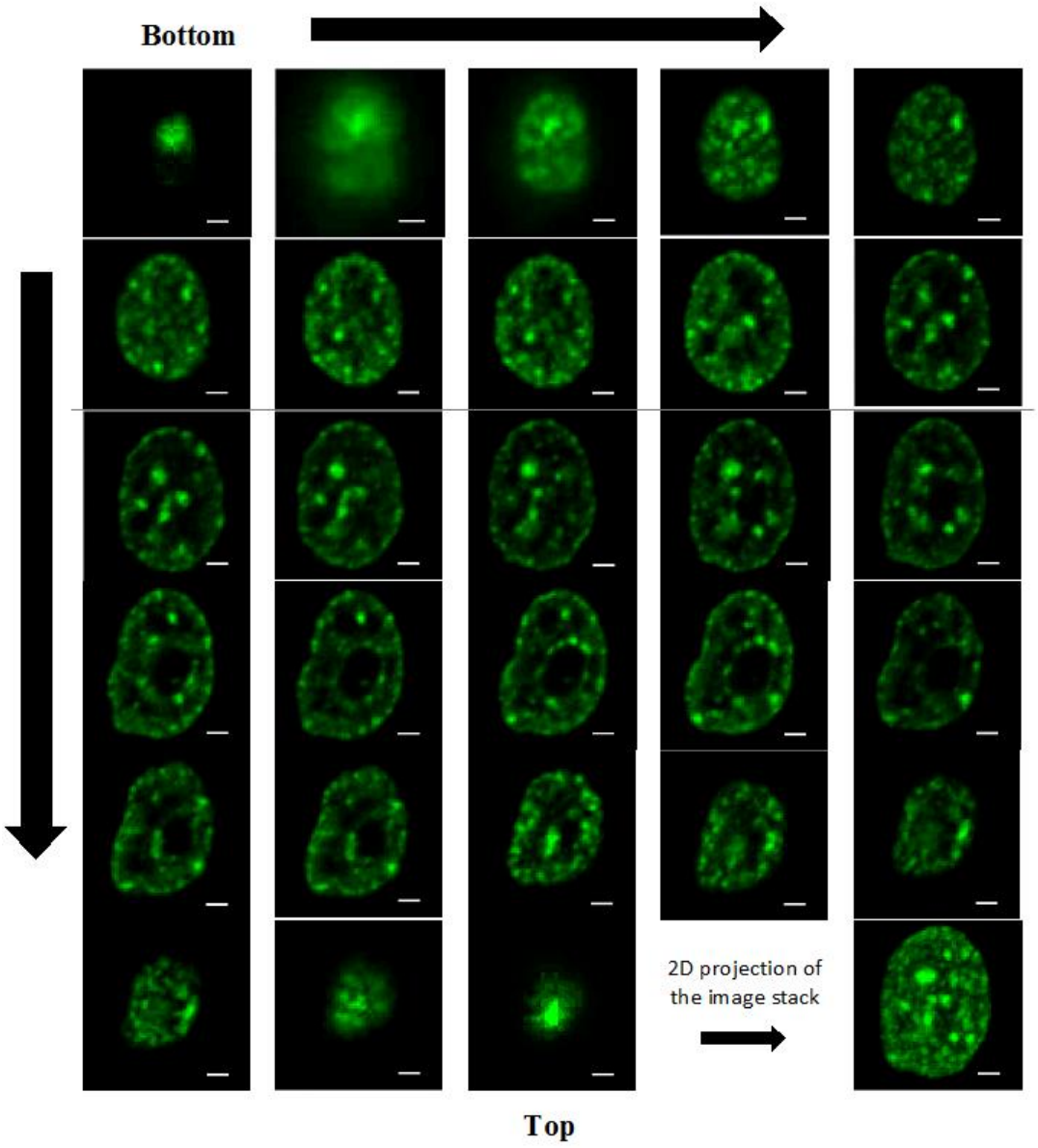


Figure S8. 2D images of a selected HEK293T cell transfected with ΔCD-ΔCSD in a z-stack. Last image shows the 2D projection of the stack. 3D reconstruction of this z-stack can be seen in the video attached in the supporting information. Scale bar = 2 μm.

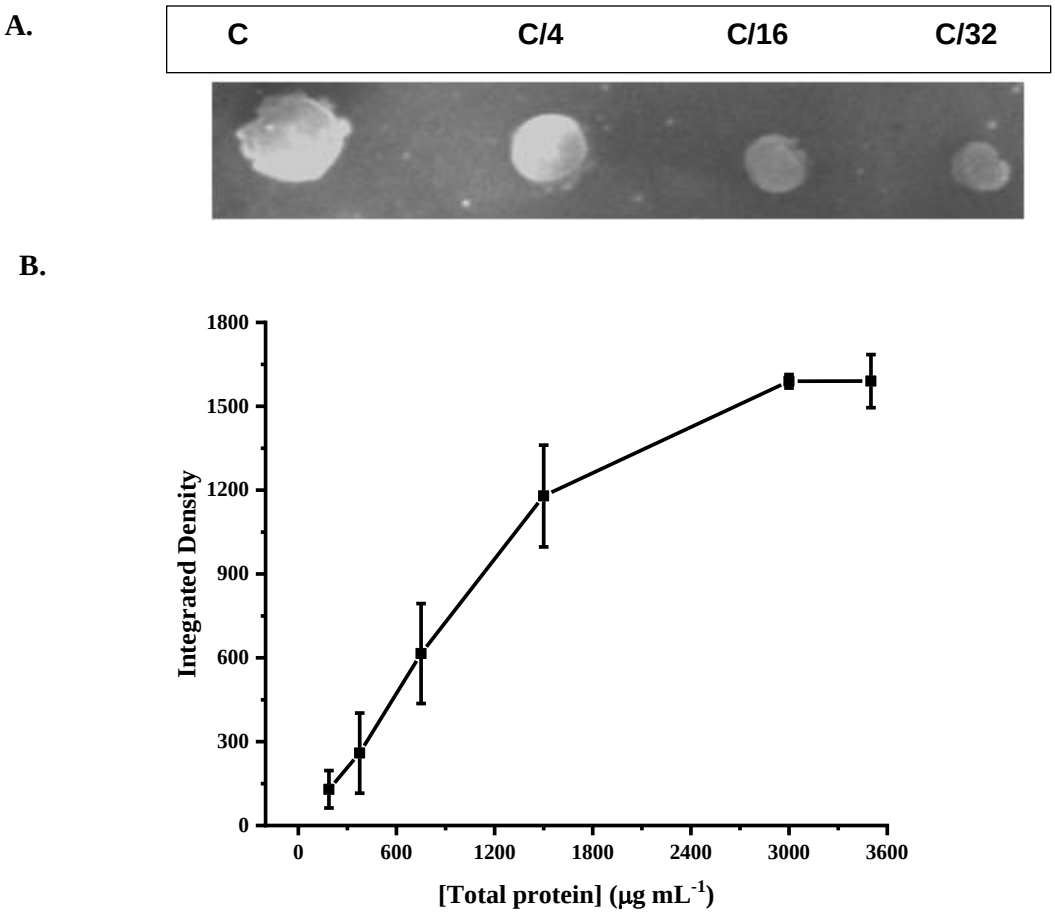


Figure S9. *In vitro* quantification of H3K9me3 via quantitative dot blot using the protein sensor ΔCD-ΔCSD-mEGFP. Representative dot blots quantifying H3K9me3 from dilution series of nuclear cell extracts (NCEs) of HEK293T cells (**A**). Integrated density of dot blots of NCEs with different protein concentration (**B**).

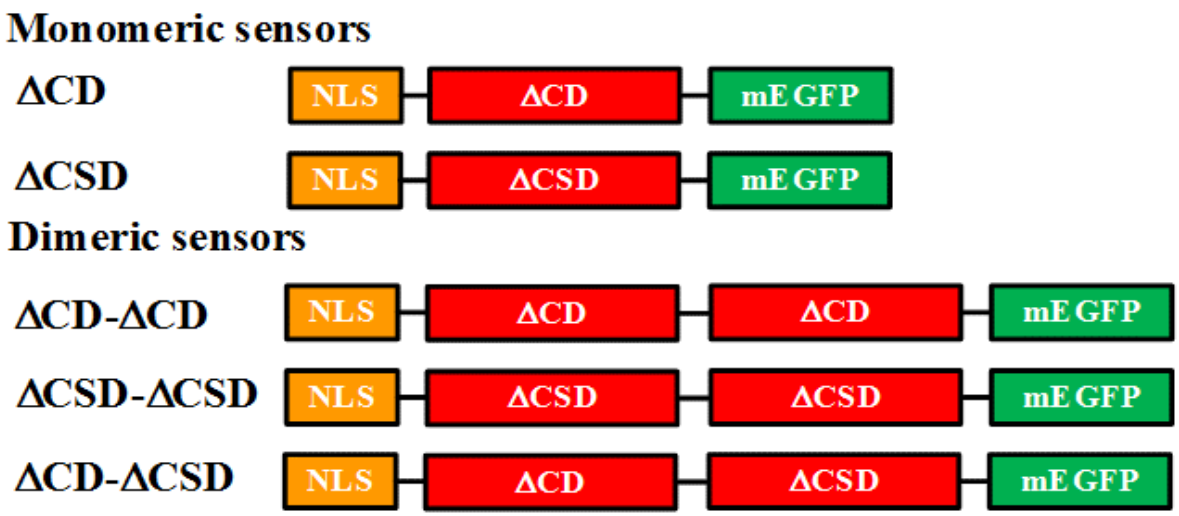


Figure S10. A graphic illustration of engineered H3K9me3 sensors consisting of different functional domains.

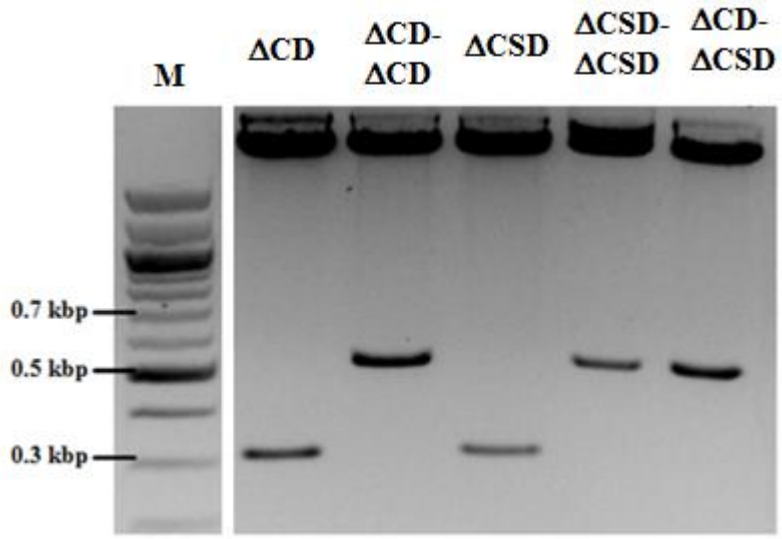


Figure S11. A 1.5% agarose gel showing the correct insert size (digested by *SacII* and *AgeI*) of pRK5 plasmids encoding the protein sensor. ΔCD, ΔCD-ΔCD, ΔCSD, ΔCSD- ΔCSD and ΔCD- ΔCSD are expected to have 305 bp, 512 bp, 308 bp, 518 bp and 515 bp, respectively.

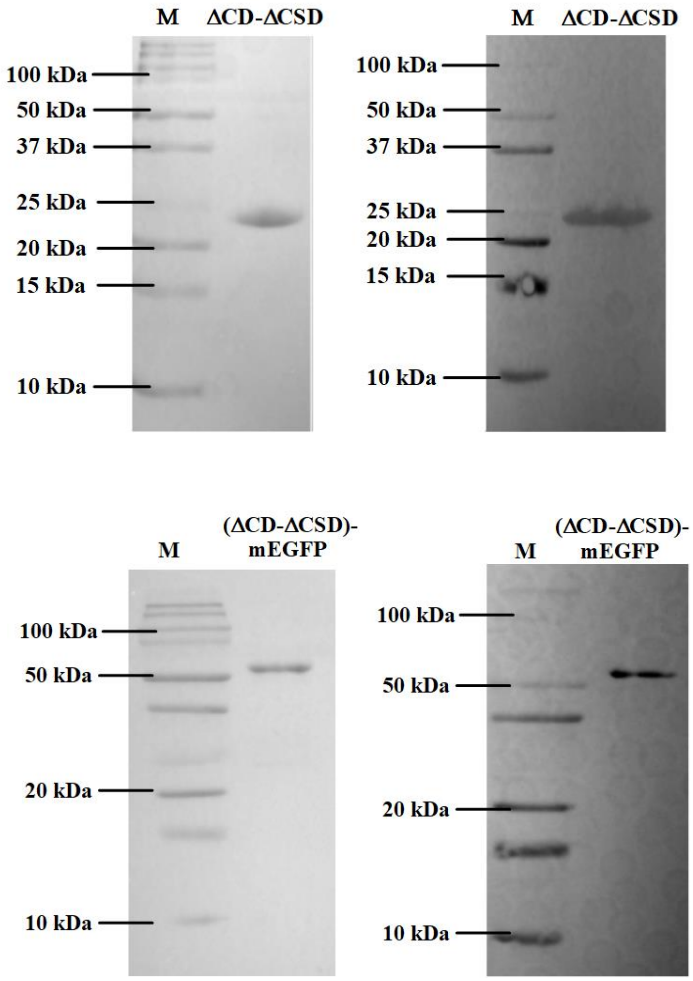


Figure S12. Recombinant ΔCD- ΔCSD (**A**) and ΔCD- ΔCSD-mEGFP (**B**) proteins. Left panels show SDS-PAGE gel (18%) of purified protein sensors after Coomassie blue staining. Right panels show Western blotting image of purified protein sensors stained with anti-His Tag (C-term) antibody (R930-25, ThermoFisher, MA, US). Expected MW of ΔCD- ΔCSD and ΔCD-ΔCSD-mEGFP is 20.1 kDa and 48.5 kDa, respectively.

Supplementary references

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4. Sanchez, O. F.; Mendonca, A.; Carneiro, A. D.; Yuan, C., Engineering Recombinant Protein Sensors for Quantifying Histone Acetylation. *ACS Sens.* **2017**.