PNAS

Supporting Information for

Binding mode-guided development of high-performance antibodies targeting site-specific posttranslational modifications

Mariapia Riso[1], Rohan N. Shah[2,3], Akiko Koide[1,4], Alexander J. Ruthenburg[3,5], Shohei Koide[1,6], Takamitsu Hattori[1,6*]

[1]Laura and Isaac Perlmutter Cancer Center, New York University Langone Health, New York, NY 10016, U.S.A.
[2]Pritzker School of Medicine, The University of Chicago, Chicago, IL 60637, U.S.A.
[3]Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL 60637, U.S.A.
[4]Department of Medicine, New York University Grossman School of Medicine, New York, NY 10016, U.S.A.
[5]Department of Biochemistry and Molecular Biology, The University of Chicago, Chicago, IL 60637, U.S.A.
[6]Department of Biochemistry and Molecular Pharmacology, New York University Grossman School of Medicine, New York, NY 10016, U.S.A.

*Correspondence to: Takamitsu Hattori
Email: Takamitsu.Hattori@nyulangone.org

This PDF file includes:

Materials and Methods

Synthetic peptides

Synthetic peptides were purchased from Abgent and Genemed Synthesis as described previously (1, 2). The phosphorylated SUMO-fusion peptides were prepared previously (3). The quality of peptides was assessed by HPLC and mass spectrometry. The sequences of the peptides used in this study are shown in Supplementary Fig. S1A. Removal of phosphorylation (i.e. dephosphorylation) of peptides was performed by incubating peptides with calf intestinal alkaline phosphatase (CIP, New England Biolabs) in rCutSmart™ Buffer (New England Biolabs) at 37°C for 1 hour.

Antibody selection using phage display

Sorting of a synthetic human antibody library was performed as described previously with modifications (4). Briefly, biotinylated PTM Fab (4-5 or 4G10) was first immobilized on Streptavidin MagneSphere Paramagnetic beads (Promega), and the empty binding sites of streptavidin were blocked with free biotin. Fab-immobilized beads were pre-incubated with an excess amount of the target peptides to allow the formation of Fab-peptide complexes. The phage library was then added to the solution containing Fab-immobilized beads in the presence of the target peptides at the saturating concentration. In the second and later rounds, phage solutions were first incubated with Fab-immobilized beads in the absence of the peptides to remove phages bound to the PTM unit alone prior to the positive selection. Phage clones bound to the complex of 4-5 and the H3K56me3 peptide were identified by phage ELISA, which was performed as described previously (4).

Antibody selection using yeast display

We constructed the yeast-display format, in which the enhancer unit is tethered to the PTM unit with a long linker to facilitate the intra-molecular dimerization (see Fig.2A). The 4-5 antibody in the scFv format was constructed previously (1), and the gene encoding 4G10 scFv was synthesized (Integrated DNA Technologies). We first constructed acceptor vectors by cloning genes encoding PTM scFv (4-5 or 4G10) attached with linkers into the pGalAga vector (5) using *Nhe*I and *Xho*I sites. The linker sequences are shown in Supplementary Fig. S2A. The genes encoding enhancer scFvs were constructed by amplifying VH and VL regions of the enhancer Fabs by PCR, and were cloned into the acceptor vectors through NEBridge® golden gate assembly (NEB) or homologous recombination. The phage pools derived from 4th round of phage selections were used for making yeast libraries. For extending the linker between 4G10 scFv and

the yeast anchor domain, Aga2, we utilized the CT linkers containing repetitive PAS and GGGS sequences reported previously (6), and cloned them into the acceptor vectors.

Yeast library sorting was performed by following the published general procedures (7). The concentration of the target peptides used for the sorting was determined based on the binding profiles of yeast libraries using the 100, 20 and 4 nM target peptides. An S3e fluorescence-activated cell sorter (Bio-Rad) was used for cell sorting.

Binding analysis using yeast surface display

The binding analysis using yeast surface display was performed as described previously (1, 2). Briefly, yeast cells sorted from the antibody library were grown at 30°C. The expression of clasping antibodies was induced by growing the cells in SG-CAA media at room temperature for 1-2 days. Binding, washing and detection were performed as described under "Peptide IP assay". The yeast cells were analyzed using an iQue Screener PLUS flow cytometer (Sartorius) or a HyperCyt screener flow cytometer (Sartorius). The median fluorescence intensity for the 75-95th percentile binding population was determined by FlowJo software (BD Biosciences) and taken as a representative signal. This sampling method of flow cytometry events minimizes contributions from events with anomalously high signals as well as events with no binding signals coming from yeast cells not expressing antibodies.

Construction, expression and purification of recombinant antibodies

The expression vector of 4-5 Fab was constructed previously (1). Genes encoding variable regions (VH and VL) of 4G10 were cloned into a Fab expression vector containing the CH1 and CL portions of the 4D5 antibody (1). The vector also contains Avi-tag at the C-terminus of the heavy chain. Fab expression and purification were performed as reported (1). Briefly, Fabs were produced in *E. coli* strain 55244 (ATCC) and purified using a HiTrap Protein G affinity column (Cytiva). The purified Fabs were biotinylated in vitro using the in-house prepared BirA enzyme in the presence of 0.5 mM Biotin and 10 mM ATP. The BirA enzyme was removed using the HiTrap Protein G affinity column (Cytiva). Purified proteins were analyzed by SDS-PAGE and size exclusion chromatography using a TSKgel SuperSW mAb HR column (Tosoh).

The long-neck scFv-Fc format was constructed as described previously (2) with modifications. We first introduced either hole mutations (Y349C, T366S, M368A, Y407V) or knob mutations (P354C, T366W) into the Fc region of mouse IgG1 to make pFUSE_mFc (hole) and pFUSE_mFc (knob) vectors. Genes encoding PTM (4-5 or 4G10) and enhancer scFvs were cloned into pFUSE_mFc (hole) and pFUSE_mFc (knob) vectors, respectively. Each vector

contains a long neck (17 amino acid linker) between the scFv and Fc regions, as described previously (2). Avi-tag and His_6-tag were attached at the C-terminus of mFc (knob) to facilitate protein purification and remove unwanted homodimer species. Expi293F cells (Thermo Fisher) were transiently co-transfected with a 1:1 mixture ratio of pFUSE_PTM scFv-mFc (hole) and pFUSE_enhancer scFv-mFc (knob) vectors using ExpiFectamine™ 293 transfection kit (Thermo Fisher) according to the manufacturer's protocol. After 1 day of incubation at 37°C with 8% CO_2, transfected cells were induced by adding ExpiFectamine™ 293 Transfection Enhancer 1 and 2 (Thermo Fisher). Induced cells were further incubated for 3 days at 37°C with 8% CO_2 (for anti-Kme3 clasping antibodies) or for 3 days at 32°C with 5% CO_2 (for anti-pY clasping antibodies). The cell culture supernatants were collected by centrifugation at 1,000 g for 20 minutes and supplemented with a protease inhibitor cocktail (Roche) and 1 mM PMSF. The supernatants were further clarified by centrifugation at 8,000 g for 30 minutes and dialyzed into phosphate buffer ((20 mM sodium phosphate (pH 7.4), 500 mM NaCl) for anti-Kme3 clasping antibodies) or TBS buffer ((50 mM Tris (pH 7.5), 500 mM NaCl) for anti-pY clasping antibodies). The supernatants were then filtrated using a 0.22 μm filter (Millipore). Long-neck scFvs were purified from the filtered supernatants using a HisTrap excel column (Cytiva). The purified proteins were biotinylated as described above, and the BirA enzyme was removed via gravitational flow using Ni Sepharose 6 Fast Flow (Cytiva). Purified proteins were analyzed by SDS-PAGE and size exclusion chromatography using a Superdex 200 increase 5/150 GL column (Cytiva).

The expression vector containing 4G10 mouse IgG was kindly gifted by Dr. Oliver Hantschel (Philipps-University Marburg). Expi293F cells (Thermo Fisher) were transiently transfected with the expression vector according to the manufacturer's protocol. Transfected cells were incubated for 7 days at 37°C with 8% CO_2. The 4G10 IgG was purified from the filtered supernatant using the HiTrap Protein G affinity column (Cytiva). Purity >90% was confirmed using SDS-PAGE.

Peptide immunoprecipitation (IP) assay

The peptide IP assay was performed as described previously (2, 8) with modifications. Briefly, 5 μL of Dynabeads™ M-280 Streptavidin (Thermo Fisher) was first washed with binding buffer (PBS containing 0.5% (w/v) BSA) twice. To prepare antibody-coated beads, 0.3 μg of biotinylated Fab, 0.15 μg of each biotinylated PTM and enhancer Fabs (i.e., a mixture of 0.15 μg PTM Fab and 0.15 μg enhancer Fab), or 0.67 μg of biotinylated long-neck scFv-Fc in binding buffer were added to the M-280 beads and incubated at 4°C for 30 minutes. The beads were then incubated with the binding buffer containing 5 μM biotin at 4°C for 5 minutes to block the empty

sites of streptavidin. This step was repeated once, and beads were suspended in the binding buffer. For recombinant commercial antibodies, AbFlex® Histone H3K27me3 antibody (Active Motif, RRID:AB_2793791, lot:29018002) and AbFlex® Histone H4K20me3 antibody (Active Motif, RRID:AB_2793777, lot:32518004) were evaluated in the peptide IP assay. To prepare antibody-coated beads, 0.5 μg of IgG was coated with 5 μL of Dynabeads™ Protein A (Thermo Fisher). The antibody-coated beads were then incubated with the biotinylated peptides at room temperature for 30 minutes. For an initial binding reaction, the binding buffer containing 5 μM biotin was used to prevent undesired binding of the biotinylated peptides to the antibody-coated streptavidin beads. When using phosphotyrosine antigens, we used TBS (50 mM Tris-HCl (pH7.5), 150 mM NaCl) instead of PBS in binding buffer, and also added a Halt™ phosphatase inhibitor cocktail (Thermo Fisher)) to binding buffer to prevent the removal of the PTM during the incubation. After washing the beads three times with washing buffer (PBS or TBS, 0.5% (w/v) BSA, 0.1% (v/v) Tween-20), the beads were incubated with Streptavidin conjugated with Dylight™ 650 (Thermo Fisher) at 4°C for 30 minutes. The beads were further washed three times and analyzed using an iQue Screener PLUS flow cytometer (Sartorius) or a HyperCyt screener flow cytometer. Data were analyzed using FlowJo software (BD Biosciences), and the signals reported are the median fluorescence intensities.

Bio-layer interferometry (BLI) experiment

The BLI experiments were performed on an Octet RED96e instrument (Sartorius). Briefly, the biotinylated H3K27me3 peptide at 2.5 nM in PBS containing 0.005% (v/v) Tween-20 was loaded on Octet® Streptavidin (SA) biosensors (Sartorius). Binding kinetics were measured against long-neck scFv-Fc antibodies at 0.4, 1.1, 3.3 and 10 nM in PBS buffer containing 0.5% (w/v) BSA, 0.005% (v/v) Tween-20 and 5 μM biotin. Data were analyzed using global fitting of a 1:1 binding model with Octet Data Analysis software (Sartorius).

Mammalian cell culture

K562 and NIH 3T3 cells were obtained as described previously (1). K562 cells were maintained in RPMI 1640 (Thermo Fisher) supplemented with 10% fetal bovine serum (FBS, Gemini Bio) and penicillin/streptomycin (Thermo Fisher) at 37°C with 5% CO_2. NIH 3T3 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, Thermo Fisher) supplemented with 10% FBS (Gemini Bio) and penicillin/streptomycin (Thermo Fisher) at 37°C with 5% CO_2. Expi293F cells (Thermo Fisher) were maintained in Expi293 Expression Medium (Thermo Fisher) at 37°C with 8% CO_2.

Western blot analysis

Western blot analysis of anti-H3K27me3 clasping antibodies using whole cell lysate of K562 cells was performed as described previously (1) with modifications. Briefly, to prepare whole cell lysate, K562 cells were washed twice with DPBS (Thermo Fisher) and suspended in 1x Laemmli SDS sample buffer containing β-mercaptoethanol followed by boiling for 5 minutes. Whole cell lysate of K562 cells (corresponding to $1x10^6$ cells) was loaded onto a 4–20% Mini-PROTEAN® TGX™ Precast Protein Gels with a 7 cm IPG/prep well (BioRad) followed by electrophoresis. The proteins were then transferred from the gel to a nitrocellulose membrane (Cytiva). The membrane was blocked with 5% (w/v) skim milk in TBST buffer (TBS containing 0.05% (v/v) Tween-20) and rinsed with TBST buffer twice. The membrane was then sandwiched into a Surf-Blot 5.5 (Thermo Fisher), a multichannel western blotting apparatus, ensuring a tight seal to avoid leakage between lanes. Long-neck scFv-Fc antibodies at the concentration of 30, 10, or 3.3 nM and anti-histone H3 polyclonal rabbit antibody (Abcam, Ab1791, RRID: AB_302613, lot GR64775-1, 1:25,000) in TBST buffer containing 1% (w/v) skim milk were added to each lane. After 1 hour of incubation with gentle shaking at room temperature, each lane was flushed with TBST buffer for 10 seconds twice. Anti-mouse Fc-HRP (Thermo Fisher, 31439, RRID: AB_228292, 1:5,000) and anti-rabbit IgG-HRP (Thermo Fisher, 31462, RRID: AB_228338, 1:10,000) were added to corresponding lanes to detect long-neck scFv-Fc and anti-histone H3 antibodies, respectively. After incubation for 1 hour at room temperature with gentle shaking, all lanes were flushed with TBST buffer twice, and then the membrane was removed from the apparatus. The membrane was further washed with TBST buffer, and incubated with Pierce™ ECL Western Blotting Substrate (Thermo Fisher). The membrane was imaged with a ChemiDoc Imager (BioRad). The images were analyzed by Image Lab software (BioRad).

For western blotting using K562 cells treated with an EZH2 methyltransferase inhibitor, K562 cells were incubated in media containing 1 μM GSK126 (Cayman Chemical) or DMSO (final 0.01% (v/v) in media) for 4 days prior to the whole cell lysate preparation. The whole cell lysate was prepared as described above, and the lysate (corresponding to approximately $3x10^4$ cells per lane) was loaded into a 4–20% Mini-PROTEAN® TGX™ Precast Protein Gels with 15 wells (BioRad) followed by electrophoresis. After transferring proteins to a nitrocellulose membrane, the membrane was blocked and washed as described above. Probing and detection procedures were performed without using the multichannel western blotting apparatus. Each membrane was probed with either 10 nM 3C1-A2, 10 nM 3C1-C2 or 3 nM 3C2-H2 long-neck scFv-Fc antibodies in TBST containing 1% (w/v) skim milk, and the antibodies were detected with anti-mouse Fc-HRP (Thermo Fisher, 31439, RRID: AB_228292, 1:5,000). The loading control was detected with

rabbit monoclonal antibody against vinculin (Thermo Fisher, 700062, RRID: AB_2532280, lot 2482648, 1:2,000) followed by anti-rabbit IgG-HRP (Thermo Fisher, 31462, RRID: AB_228338, 1:10,000).

For the western blot analysis of anti-pY clasping antibodies, approximately 10 ng of SUMO-fused peptide was loaded into each lane of the precast protein gel. To prepare whole cell lysate, NIH 3T3 cells were grown to approximately 90% confluency and serum-starved by replacing media with DMEM containing 0.1% FBS and incubating at 37°C with 5 % CO_2 for 20 hours. The cells were then incubated in DMEM containing 0.1% FBS and 100 ng/mL PDGF-BB (Cell Signaling Technology, 50611) for 10 minutes at 37°C to induce PDGFRb-mediated signaling. After washing the cells with DPBS, the cells were lysed by adding 2x Laemmli SDS sample buffer containing β-mercaptoethanol. The whole cell lysate was collected and boiled for 5 minutes. The whole cell lysate of K562 was prepared as described above.

Western blot was performed as described above, except that PVDF membrane (Millipore) was used for western blot analysis of anti-PDGFRb pY716 clasping antibodies using whole cell lysates (Fig. 4C, Supplementary Fig. S8F). For western blot analysis using SUMO-fusion peptides, each membrane was probed with 2 nM long-neck scFv-Fc antibodies or 2 nM 4G10 IgG. For western blot analysis using whole cell lysates, the multichannel western blotting apparatus was used as described above. After assembling the multichannel apparatus, calf intestinal alkaline phosphatase (CIP, New England Biolabs) diluted in rCutSmart™ Buffer (New England Biolabs) was added to the corresponding lanes and incubated at 37°C for 1 hour for dephosphorylation. All other lanes were incubated with TBST buffer. Membranes were then probed with 5 nM long-neck scFv-Fc antibodies, 5 or 2 nM 4G10 IgG, anti-vinculin rabbit antibody (Thermo Fisher, 700062, RRID: AB_2532280, lot 2482648, 1:2,000), anti-PDGFRb rabbit antibody (Cell Signaling Technology, 3169, RRID: AB_2162497, lot 13, 1:1,000), anti-BCR rabbit antibody (Cell Signaling Technology, 3902,RRID: AB_2063777, lot 7, 1:500), or anti-BCR pY177 rabbit antibody (Cell Signaling Technology, 3901, RRID: AB_2063779, lot 9, 1:500). The antibodies were detected using either anti-mouse Fc-HRP (Thermo Fisher, 31439, RRID: AB_228292, 1:5,000) or anti-rabbit IgG-HRP (Thermo Fisher, 31462, RRID: AB_228338, 1:10,000).

Immunoprecipitation followed by Western blot (IP-Western)

NIH 3T3 cells were serum-starved and stimulated as described under “Western blot analysis”. After 10 minutes of incubation at 37°C in the presence or absence of PDGF-BB (Cell Signaling Technology, 50611), the cells were washed with ice-cold DPBS once. NIH 3T3 cells were then lysed in ice-cold RIPA buffer (50 mM Tris (pH 8.0), 150 mM NaCl, 5 mM EDTA, 1%

(v/v) NP-40, 0.1% (w/v) SDS) supplemented with a cOmplete™ protease inhibitor cocktail (Roche) and a Halt™ phosphatase inhibitor cocktail (Thermo Fisher) on ice for 20 minutes with occasional swirling of the plates. Cell lysates were collected and centrifuged at 15,000 rpm for 15 minutes. The supernatant was collected and measured for a total protein amount using a Micro BCA™ protein Assay Kit (Thermo Fisher).

To prepare antibody-immobilized beads, 5 μL of Dynabeads™ M-280 streptavidin (Thermo Fisher) was washed with bead buffer (TBS, 0.1 % (w/v) BSA, 0.02% Tween-20) twice. Then, 0.67 μg of biotinylated long-neck scFv-Fc antibody was incubated with the beads at 4°C for 1 hour. The beads were incubated with the bead buffer containing 10 μM biotin at 4°C for 15 minutes to block the empty sites of streptavidin. The beads were washed with bead buffer twice and then with IP buffer (25 mM Tris (pH 7.2), 150 mM NaCl, 1 mM EDTA, 1% (v/v) NP-40, 5% (v/v) glycerol). The lysate containing 500 μg of proteins was first incubated with 5 μL of unimmobilized M-280 beads to remove proteins that nonspecifically bind to the beads. The supernatant (i.e. pre-cleared lysate) was then mixed with the antibody-coated beads for 1 hour at 4°C with rotation. The beads were then incubated with IP buffer for 10 minutes at 4°C with rotation. This step was repeated four times, followed by an additional washing step with TBS buffer twice. The 2x Laemmli SDS sample buffer containing β-mercaptoethanol was added to the beads and boiled for 5 minutes. The supernatant was isolated using a magnetic stand, and loaded into a 4–20% Mini-PROTEAN® TGX™ Precast Protein Gels with 15 wells (BioRad) followed by electrophoresis. The 1/10 amount of the input sample was used as the control. Western blot was performed as described under “Western blot analysis”. After the blocking procedure, the PVDF membrane was probed with anti-PDGFRb rabbit monoclonal antibody (Cell Signaling Technology, 3169, RRID: AB_2162497, lot 13, 1:1,000) and detected with anti-rabbit IgG-HRP (Thermo Fisher, 31462, RRID: AB_228338, 1:10,000).

Internal Standard Calibrated ChIP (ICeChIP)

ICeChIP was conducted as previously described (9-11), with modifications. Briefly, K562 cell pellets were washed twice with 5 mL of PBS, then washed twice with 5 mL of Buffer N (15 mM Tris-HCl (pH 7.5), 15 mM NaCl, 60 mM KCl, 8.5% (w/v) Sucrose, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM DTT, 200 μM PMSF, 50 μg/mL BSA, 1x Protease Inhibitor Cocktail [1 mM AEBSF, 0.8 μM aprotinin, 20 μM leupeptin, 15 μM pepstatin A, 40 μM bestatin, 15 μM E-64; from 200x in DMSO]), with each wash consisting of resuspension of the cell pellet, centrifugation at 500 g for 5 minutes at 4°C, and removal of supernatant. The four-times washed pellet was resuspended into 2 packed cell volumes of Buffer N. One volume of Lysis Buffer (Buffer N supplemented with 0.6% (v/v) NP-

40 substitute) was then added (to a final concentration of 0.3% (v/v) NP-40 substitute) and incubated on ice for 10 minutes to lyse cells. The crude nuclei were then centrifuged at 500 g for 5 minutes at 4°C and resuspended in at least 6 packed nuclear volumes of Buffer N. This crude nuclei suspension was then applied atop 7.5 mL of Sucrose Cushion N (15 mM Tris-HCl (pH 7.5), 15 mM NaCl, 60 mM KCl, 30% (w/v) Sucrose, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM DTT, 200 μM PMSF, 50 μg/mL BSA, 1x Protease Inhibitor Cocktail) in a 15 mL tube before being centrifuged at 500 g for 12 minutes at 4°C in a swinging-bucket rotor. The supernatant was then discarded, and the purified nuclei were resuspended in approximately two packed nuclear volumes of Buffer N.

Nucleic acid content of this suspension was then quantified as previously described (9-11). For each of the four replicates, 2 μL of the nuclei suspension was added to 48 μL of 2 M NaCl to lyse nuclei, and the chromatin was solubilized by water-bath sonication until the suspension could be easily pipetted. Nucleic acid content was then measured by Nanodrop, and the nuclei suspension was diluted with Buffer N to achieve a concentration of 1 μg/μL of nucleic acid. This suspension was then dispensed into 100 μL aliquots, flash-frozen, and stored at -80°C until the time of use.

At the time of use, a single aliquot was thawed and spiked with approximately 2 μL of each barcoded nuclei standard (~2 nM total concentration for all members of each ladder). The suspension was then mixed well and heated on a thermoshaker to 37°C for 2 minutes. Micrococcal nuclease digestion was then conducted to generate mononucleosomes, in which 23 Worthington Units of pre-warmed micrococcal nuclease (MNase, Worthington) was added to the heated nuclei suspension. Samples were then incubated at 37°C while shaking at 900 rpm on a thermoshaker for 12 minutes. Digestion was stopped by adding 1/9 volume of filter-sterilized 10x MNase Stop Buffer (100 mM EDTA, 100 mM EGTA) while slowly vortexing. Nuclei were then lysed by adding 5 M NaCl dropwise to a final concentration of 600 mM while slowly vortexing. The lysed nuclei were then centrifuged at 18,000 g for 1 minute to pellet insoluble debris (typically a barely visible white pellet), and the soluble fraction was recovered and saved.

Nucleosomes were then purified by HAP purification, as previously described (9-11). Sixty-six mg of HAP resin (BioRad, CHT Ceramic Hydroxyapatite, Type I, 20 μm particle) was rehydrated with 200 μL of filter-sterilized HAP Buffer 1 (5 mM Sodium Phosphate (pH 7.2), 600 mM NaCl, 1 mM EDTA, 200 μM PMSF). The soluble component of the lysed nuclei was then added to the rehydrated HAP resin and incubated on a rotator for 10 minutes at 4°C. The HAP slurry was then added to a centrifugal filter unit (Millipore, Ultrafree MC HV Centrifugal Filter 0.45

μm pore size) and spun at 1,000 g for 30 seconds at 4°C. The HAP resin was then washed four times with 200 μL of HAP Buffer 1 and four times with 200 μL of HAP Buffer 2 (5 mM Sodium Phosphate (pH 7.2), 100 mM NaCl, 1 mM EDTA, 200 μM PMSF), with each wash consisting of adding the buffer to the resin and spinning at 1,000 g for 30 seconds at 4°C. Chromatin was eluted from HAP resin into a clean tube with three spins each with 100 μL of filter-sterilized HAP Elution Buffer (500 mM Sodium Phosphate (pH 7.2), 100 mM NaCl, 1 mM EDTA, 200 μM PMSF). The nucleic acid concentration of the elution was then quantified by Nanodrop, and the concentration of the elution was adjusted to 20 ng/uL with filter-sterilized ChIP Buffer 1 (25 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 100 mM KCl, 10% (v/v) glycerol, 0.1% (v/v) NP-40 Substitute).

Anti-H3K27me3 commercial antibody (Cell Signaling Technology, 9733, clone C36B11, RRID: AB_2616029, lot 19) was conjugated to protein G beads as previously described (9-11). Briefly, 10 μL of Protein G Dynabeads (Thermo Fisher) was washed on a magnetic rack with 50 μL of ChIP Buffer 1 and resuspended in 150 μL of ChIP Buffer 1. Into this buffer, 1.2 μg of antibody was added, and the antibody and Dynabeads were allowed to conjugate for 1 hour at 4°C on a rotator. After incubation, the antibody-coated beads were washed with 200 μL of ChIP Buffer 1 and resuspended into 50 μL of ChIP Buffer 1. Anti-H3K27me3 clasping Fabs were prepared in parallel. To prepare Fab-immobilized beads, 0.5 μg of each biotinylated 4-5 Fab and 3C2-H2 Fab were added to 150 μL of ChIP Buffer 1, and 20 μL of Streptavidin M-280 Dynabeads (Thermo Fisher) were washed with 100 μL of ChIP Buffer 1 and resuspended into 50 μL of ChIP Buffer 1. The Fab solution was then added to the Dynabead solution and incubated for 1 hour at 4°C on a rotator. After incubation, the beads were removed by a magnetic rack and resuspended into 200 μL of ChIP Buffer 1 with 5 μM biotin, then allowed to incubate for 10 minutes at 4°C on a rotator. This biotin wash was then repeated once more. After the second such incubation, the beads were resuspended into 50 μL of ChIP Buffer 1.

ICeChIP was conducted as previously described (9-11). HAP-purified chromatin (800 ng in 40 μL) was added to each of the Dynabead suspensions and incubated for 15 minutes at 4°C on a rotator. After this, beads were washed twice with ChIP Buffer 2 (25 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 300 mM KCl, 10% (v/v) glycerol, 0.1% (v/v) NP-40 substitute) and once with ChIP Buffer 3 (10 mM Tris-HCl (pH 7.5), 250 mM LiCl, 1 mM EDTA, 0.5% (w/v) Sodium Deoxycholate, 0.5% (v/v) NP-40 substitute), with each wash consisting of resuspension into 150 μL of buffer by use of magnetic rack, transfer to a new siliconized tube, and incubation for 10 minutes at 4°C on a rotator. After these washes, the beads were resuspended in ChIP Buffer 1, transferred to a new siliconized tube, rinsed with TE buffer on a magnetic rack, and resuspended into 50 μL of ChIP

Elution Buffer (50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 1% (w/v) SDS, Filter Sterilized). Samples were then heated to 55°C for 5 minutes, and the supernatant was saved as the ChIP elutions. To prepare the input sample, 15 μL of the HAP-purified nucleosomes were also added to 35 μL of ChIP Elution Buffer. Each of the ChIP elution and the input were supplemented by 2 μL of 5 M NaCl, 1 μL of 500 mM EDTA, and 1 μL of 10 mg/mL Proteinase K. Samples were then digested at 55°C for 2 hours. After digestion, DNA was purified by adding 75 μL of Ampure XP (Beckman Coulter), and incubating at room temperature for 15 minutes. The beads were then collected on a magnetic rack and, while on the rack, the beads were rinsed twice with 150 μL of 70% ethanol without resuspending beads. Beads were then dried and resuspended into 50 μL of ddH2O, which was then recovered and stored at -20°C.

Illumina Library Preparation and Sequencing

Illumina libraries were prepared as previously described (9-11). Briefly, DNA was quantified using Quant-iT PicoGreen (Thermo Fisher) per manufacturer instruction. Libraries were then generated from up to 10 ng of each DNA sample (input or IP) with the NEBNext® Ultra™ II DNA Library Prep Kit (New England Biolabs) per manufacturer instructions. Libraries were then quantified and pooled for sequencing on a NovaSeq (Illumina) per standard protocols by the University of Chicago Functional Genomics Facility.

Quantification and Statistical Analysis

Sequencing reads were aligned as previously described (9-11). Briefly, a reference genome was created consisting of the human genome (hg38) appended by the sequences of each of the nucleosome standard barcodes. Reads were then aligned, filtered, parsed into genome coverage bedgraphs, and computed as histone modification density (HMD) bedgraphs by use of the scripts previously published for this purpose (11). For the barcode analyses, the LINEST function in MS Excel was used for linear regression analyses, and the IGV browser4 was used to generate locus tracks of histone modification density.

Sample Normalization and Antibody Profiling Chromatin Immunoprecipitation (SNAP-ChIP)

The isolation of nuclei from K562 cells and HAP purification of nucleosomes were performed as described under “ICeChIP”, except that we spiked SNAP-ChIP® K-MetStat Panel (EpiCypher) into purified nuclei (1 μL SNAP-ChIP panel to 5 μg sample chromatin) prior to MNase

digestion, by following the manufacturer's instruction. To prepare antibody-coated beads, 2.2 μg of biotinylated 3C2-H2 long-neck scFv-Fc was coated to 40 μL of Streptavidin M-280 Dynabeads (Thermo Fisher). For the commercial antibody, 3 μg of AbFlex® Histone H3K27me3 antibody (Active Motif, RRID:AB_2793791, lot:29018002) was coated to 12.5 μL of Dynabeads™ Protein A (Thermo Fisher). The antibody coating procedure was the same as described under "ICeChIP". Long-neck scFv-Fc is compatible with Protein A or Protein G beads, but we prefer using streptavidin beads due to the strong interaction between biotin and streptavidin to minimize the dissociation of the antibody from the beads during the IP procedures. The antibody-immobilized beads were then mixed with 3 μg of HAP-purified chromatin and incubated at 4°C on a rotator for 1 hour. Washing, elution and digestion with Proteinase K were performed as described under "ICeChIP". After digestion, DNA was purified using QIAquick PCR Purification Kit (Qiagen).

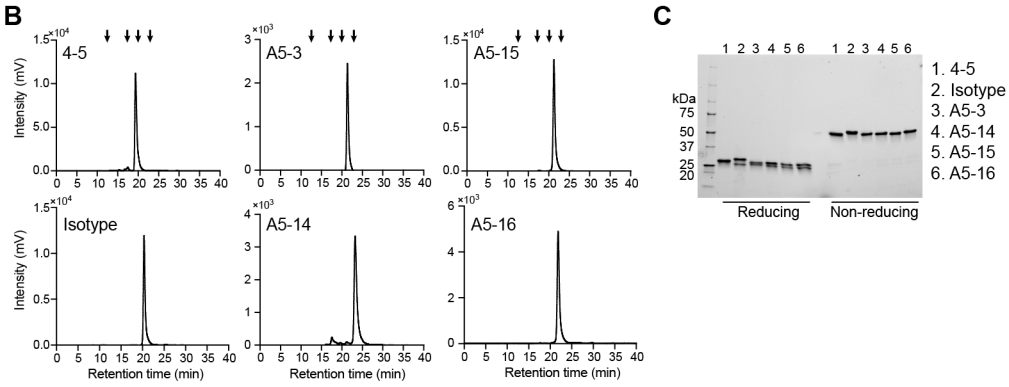
Quantitative PCR (qPCR)

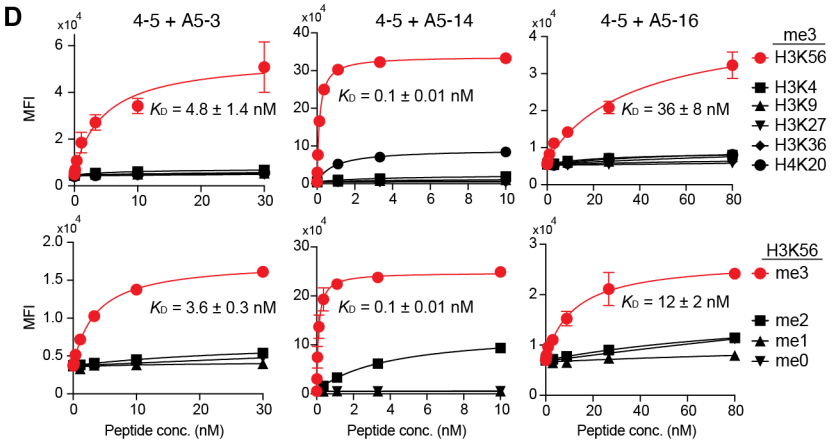
qPCR of purified DNA was performed according to EpiCypher's instructions using SsoAdvanced™ Universal SYBR® Green Supermix (BioRad) with 5 μM SNAP-ChIP qPCR primers (EpiCypher) in 20 μL reaction volume. A CFX96 Touch Real-Time PCR Detection System (BioRad) was used for qPCR, and data were analyzed using CFX Maestro software (BioRad). Antibody specificity (off-target relative to on-target) and enrichment (% on-target input recovered) were calculated according to EpiCypher's instructions.

A

Name	Sequence
H3K4	ARTKQTARKSTG-GY-K(biotin)-D
H3K9	ARTKQTARKSTGGKAP-GY-K(biotin)-D
H3K27	QLATKAARKSAPATGG-GY-K(biotin)-D
H3K36	SAPATGGVKKPHRYRP-GG-K(biotin)-D
H3K56	LREIRRYQKSTELLIR-GG-K(biotin)-D
H3K79	VREIAQDFKTDLRFQS-GY-K(biotin)-D
H4K20	KGGAKRHRKVLRDNIQ-GY-K(biotin)-D

Name	Sequence
PTPN11 pY580	DSARVpYENVGLMQ-GG-K(biotin)-D
BCR pY177	AEKPFpYVNVEFHH-GG-K(biotin)-D
FRS2 pY196	EQVHTpYVNTTGVQ-GG-K(biotin)-D
PDGFRb pY716	PSAELpYSNALPVG-GG-K(biotin)-D



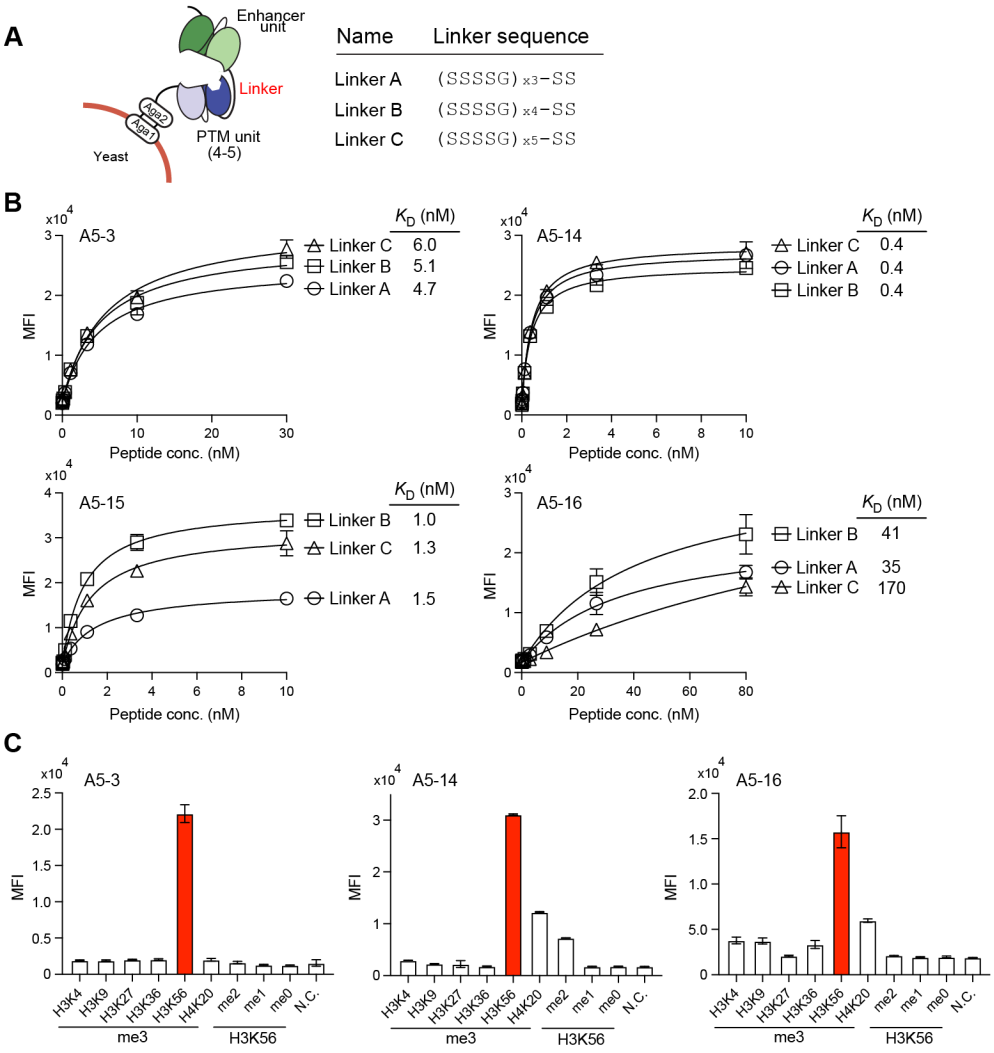


E

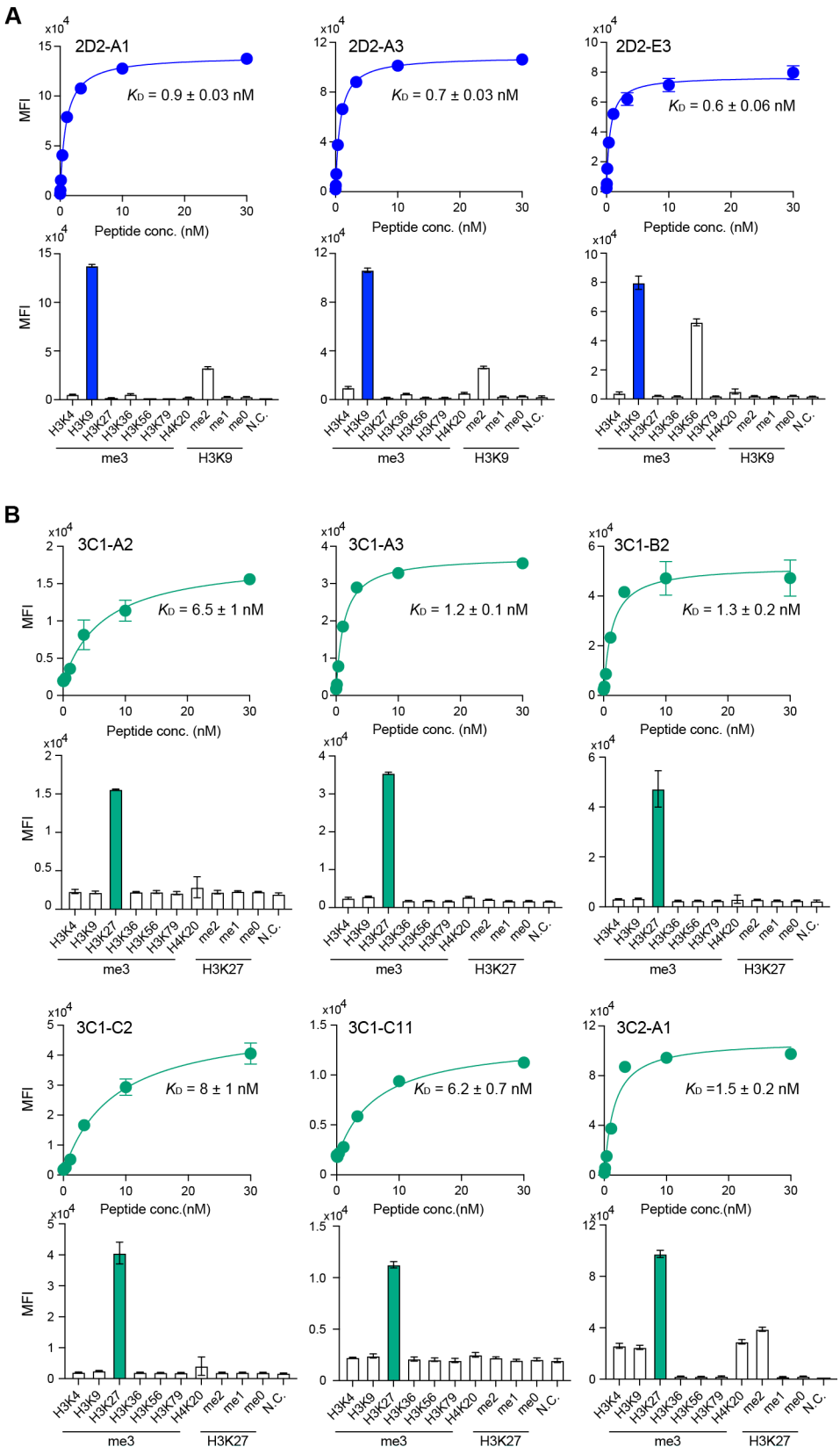
Clone	H1	H2	H3	L3
A5-3	ISYSYIH	SIYSYSGYTSYADSVKG	YYGYKSKIAMDY	QQESQQLIT
A5-14	VYYSSIH	YIYPYSGYTSYADSVKG	DSYKPFGWGLDY	QQSSFSELVT
A5-15	IYYSYIH	SIYSYSGYTSYADSVKG	QYGEVKKYGWGLDY	QQSGAYDYLFT
A5-16	VYSSSIH	YIYPYSGYTSYADSVKG	SFFYPYVKGGMAMDY	QQASSGSWPLT

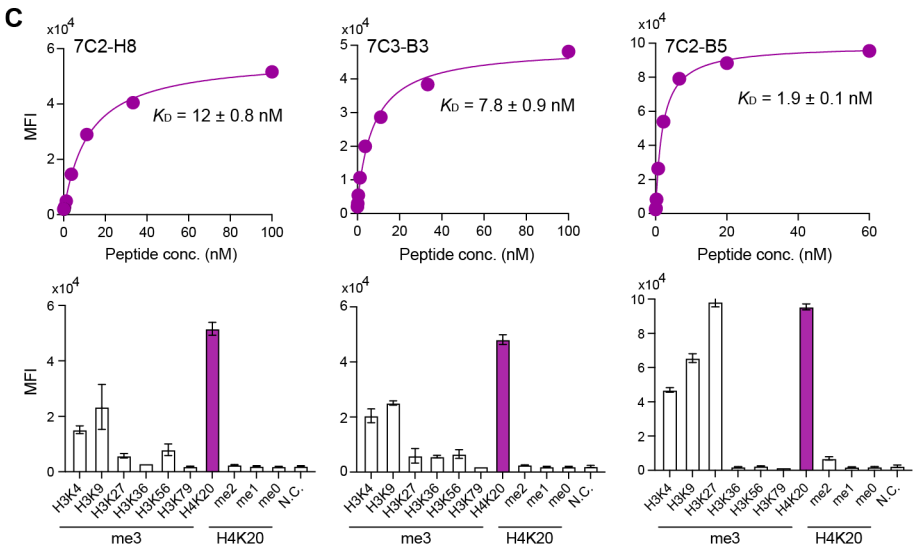
Supplementary Figure S1. Amino acid sequences of antigen peptides and characterization of clasping Fabs targeting H3K56me3. (A) The amino acid sequences of histone peptides (left table) and pY peptides (right table) in this study. The residues harboring PTMs are labeled in red. The lysine residue at the GYKD or GGKD tags is biotinylated. (B and C) Size-exclusion chromatograms using a TSKgel SuperSW mAb HR column (B) and SDS-PAGE profiles (C) of purified Fabs. The elution positions of standard proteins (with molecular weights of 670, 158, 44, and 17 kDa, respectively) are indicated by arrows in (B). (D) The binding titration curves of clasping Fabs against the target and off-target peptides with the peptide IP assay. The PTM and enhancer Fabs were co-immobilized on beads (see Materials and Methods). The apparent K_D values, calculated from the curve fitting of a 1:1 binding model to the target peptide are shown. Sequence specificity (top panels) and methylation-state specificity (bottom panels) are assessed.

Data shown here are from triplicate measurements. Error bars indicate the s.d. (E) CDR sequences of enhancer antibodies. Residues that are randomized in the antibody library but located outside CDRH1 are indicated by an underscore.



Supplementary Figure S2. Linker optimization and binding characterization of clasping antibodies to H3K56me3 in the yeast-display format. (A) The length of the linker shown in red in the left cartoon was optimized. The right table shows the amino acid sequences of the linkers we tested. (B) Binding titration curves of clasping antibodies to H3K56me3 harboring different linker lengths in the yeast display format. The apparent K_D values were calculated from the curve fitting of a 1:1 binding model. (C) Specificity test of clasping antibodies to H3K56me3 in the yeast-display format harboring the 22-residue linker (Linker B). The concentrations of the peptides used for A5-3, A5-14, and A5-16 were 30, 10 and 80 nM, respectively. Data shown here are from triplicate measurements. Error bars indicate the s.d.

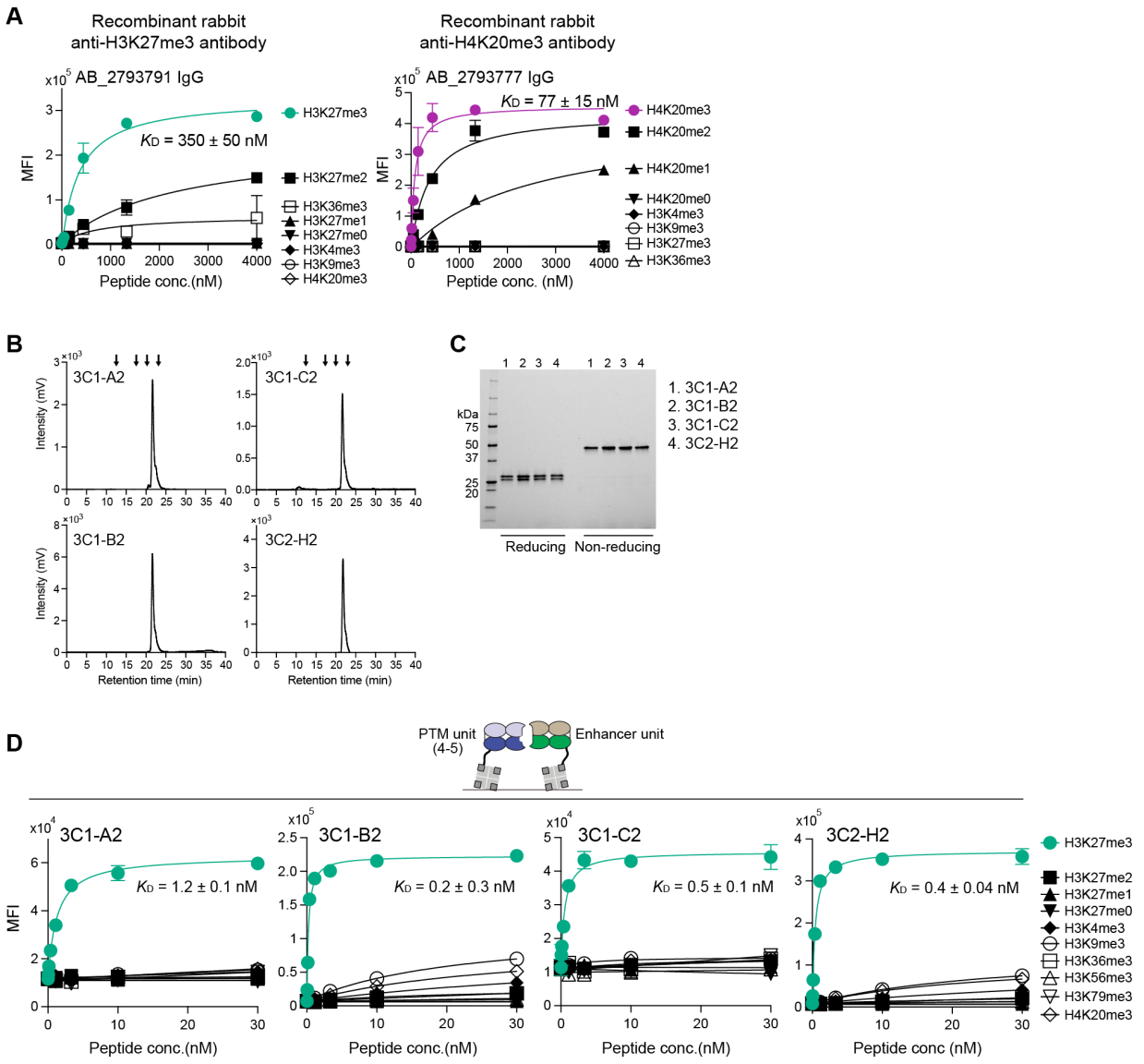

A
2D2-A1
KD = 0.9 ± 0.03 nM
2D2-A3
KD = 0.7 ± 0.03 nM
2D2-E3
KD = 0.6 ± 0.06 nM
MFI
Peptide conc. (nM)
H3K4 H3K9 H3K27 H3K36 H3K56 H3K79 H4K20 me2 me1 me0 N.C.
me3
H3K9
B
3C1-A2
KD = 6.5 ± 1 nM
3C1-A3
KD = 1.2 ± 0.1 nM
3C1-B2
KD = 1.3 ± 0.2 nM
H3K27
3C1-C2
KD = 8 ± 1 nM
3C1-C11
KD = 6.2 ± 0.7 nM
3C2-A1
KD =1.5 ± 0.2 nM
Peptide conc.(nM)



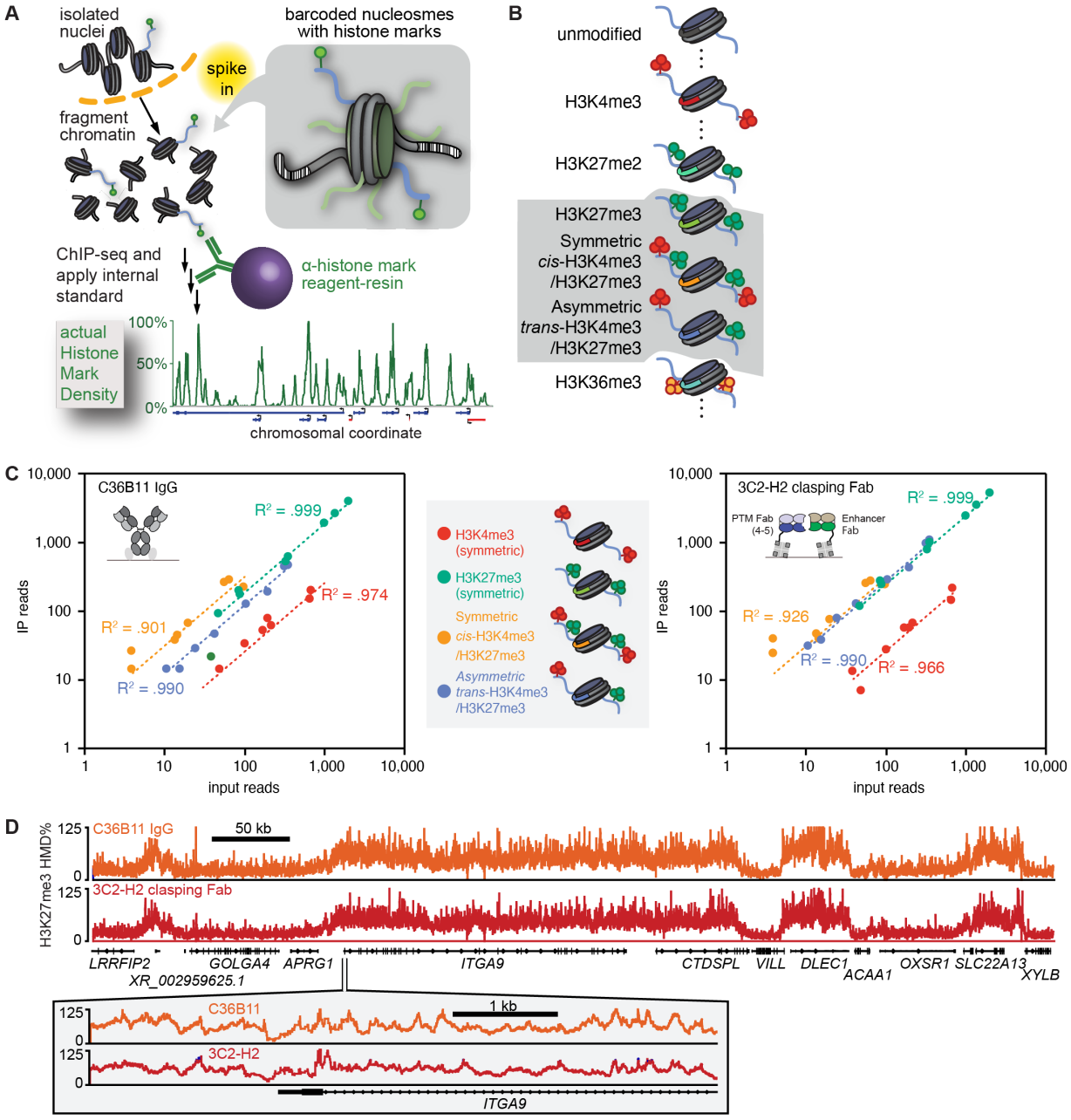
D

Clone	H1	H2	H3	L3
2D2-A1	IYYYSIH	SIYPYSGYTSYADSVKG	SYEWWSWPSYGMDY	QQSSSSLIT
2D2-A3	IYYSSIH	SIYPYSGYTSYADSVKG	SYEWWSWPSYGMDY	QQSSSWLPIT
2D2-E3	IYYYSIH	SIYPYSGYTSYADSVKG	EGMNWGLDY	QQYTYYPIT
2D2-H3	VYYSSIH	SIYSYSGYTSYADSVKG	SYEWWSWPSYGMDY	QQSSRNGPVT
3C1-A2	IYSSSIH	YIYSYSGYTSYADSVKG	EASYGRYYVPALDY	QQSSGYDFPIT
3C1-A3	IYSSSIH	YIYSYSGYTSYADSVKG	EASYGRYYVPALDY	QQYTGKKYLIT
3C1-B2	IYSSSIH	YIYSYSGYTSYADSVKG	EASYGRYYVPALDY	QQSSSSLIT
3C1-C2	LSSSSIH	YIYSYSGYTSYADSVKG	EASYGRYYVPALDY	QQSSSSLIT
3C1-C11	IYSSSIH	YIYSYSGYTSYADSVKG	EASYGRYYVPALDY	QQGYGWLVT
3C2-A1	LYYSSIH	YIYPYSGYTSYADSVKG	EASYGRYYVPALDY	QQSSSSLIT
3C2-H2	IYSSSIH	YIYSYSGYTSYADSVKG	EASYGRYYVPALDY	QQSYSSLIT
7C2-H6	IYSSSIH	YIYPYSGYTSYADSVKG	EFYYSDTGMDY	QQSSYSYPIT
7C2-H8	LYSSSIH	YIYPYSGYTSYADSVKG	EFYYSDTGMDY	QQSSYSYPIT
7C3-B3	VYSSSIH	YIYPYSGYTSYADSVKG	EFYYSDTGMDY	QQSSYSYPIT
7C2-B5	IYSSSIH	YIYPYSGYTSYADSVKG	DWPSWRYSAMDY	QQSSYSYPIT

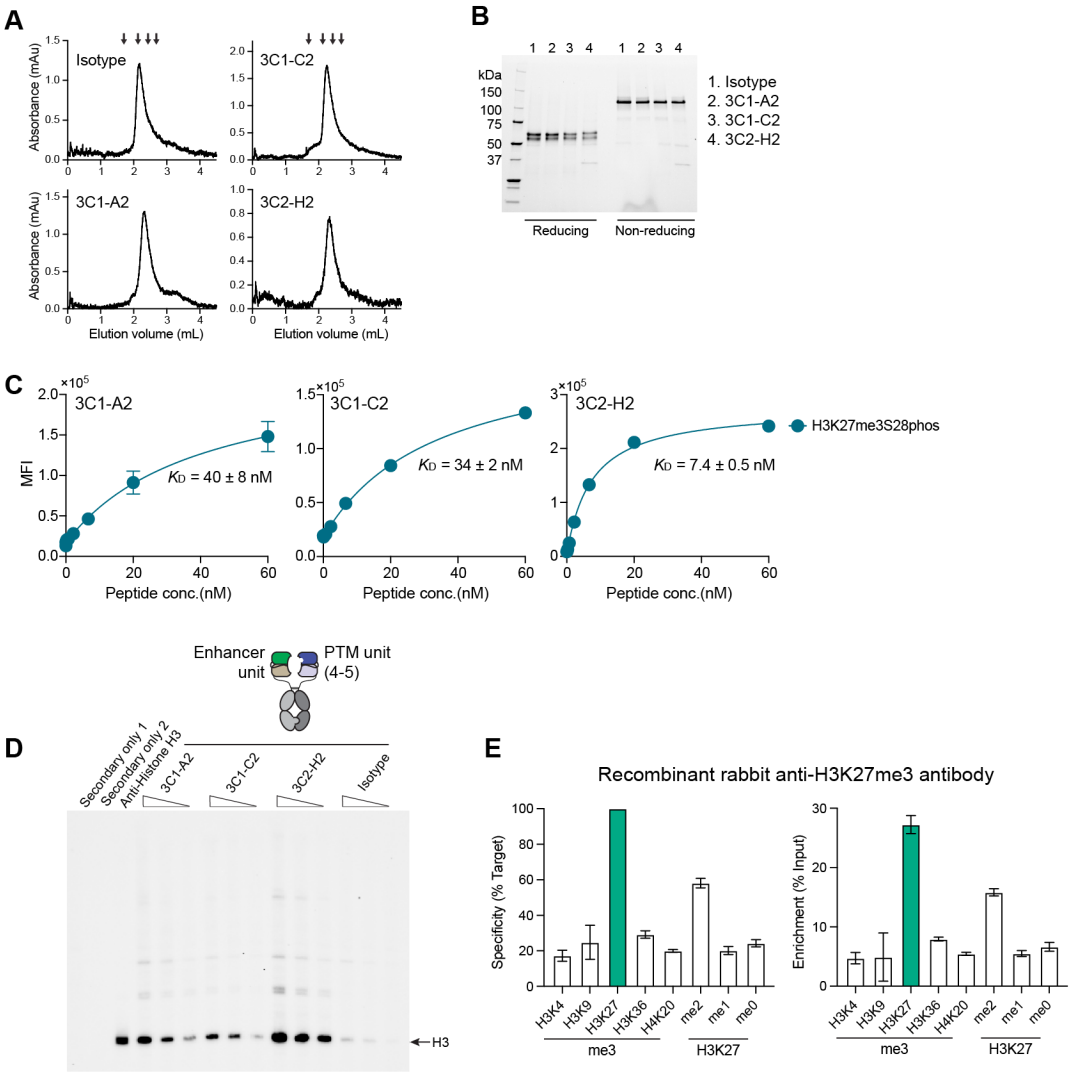
Supplementary Figure S3. Characterization of clasping antibodies in the yeast-display format. (A-C) The binding characterization of clasping antibodies targeting H3K9me3 (A), H3K27me3 (B), and H4K20me3 (C). The top panels show the binding titration curves to the target peptides. The apparent K_D values were calculated from the curve fitting of a 1:1 binding model. The bottom panels show the specificity test using multiple histone peptides. The highest peptide concentrations used in the binding titration curves were utilized in the specificity test. Data shown here are from triplicate measurements. Error bars indicate the s.d. (D) CDR sequences of enhancer antibodies. Residues that are randomized in the antibody library but located outside CDRH1 are indicated by an underscore.



Supplementary Figure S4. Characterization of commercial recombinant antibodies and anti-H3K27me3 clasping antibodies with the peptide IP assay. (A) Binding titration of commercially available recombinant antibodies to H3K27me3 (left) and H4K20me3 (right). (B and C) Size-exclusion chromatograms using a TSKgel SuperSW mAb HR column (B) and SDS-PAGE profiles (C) of purified Fabs. The elution positions of standard proteins (with molecular weights of 670, 158, 44, and 17 kDa, respectively) are indicated by arrows in (B). (D) Binding titration of clasping Fabs. The PTM and enhancer Fabs were co-immobilized on beads (see Materials and Methods). The apparent K_D values calculated from the curve fitting of a 1:1 binding model to the target peptide are shown. Data shown here are from triplicate measurements. Error bars indicate the s.d.

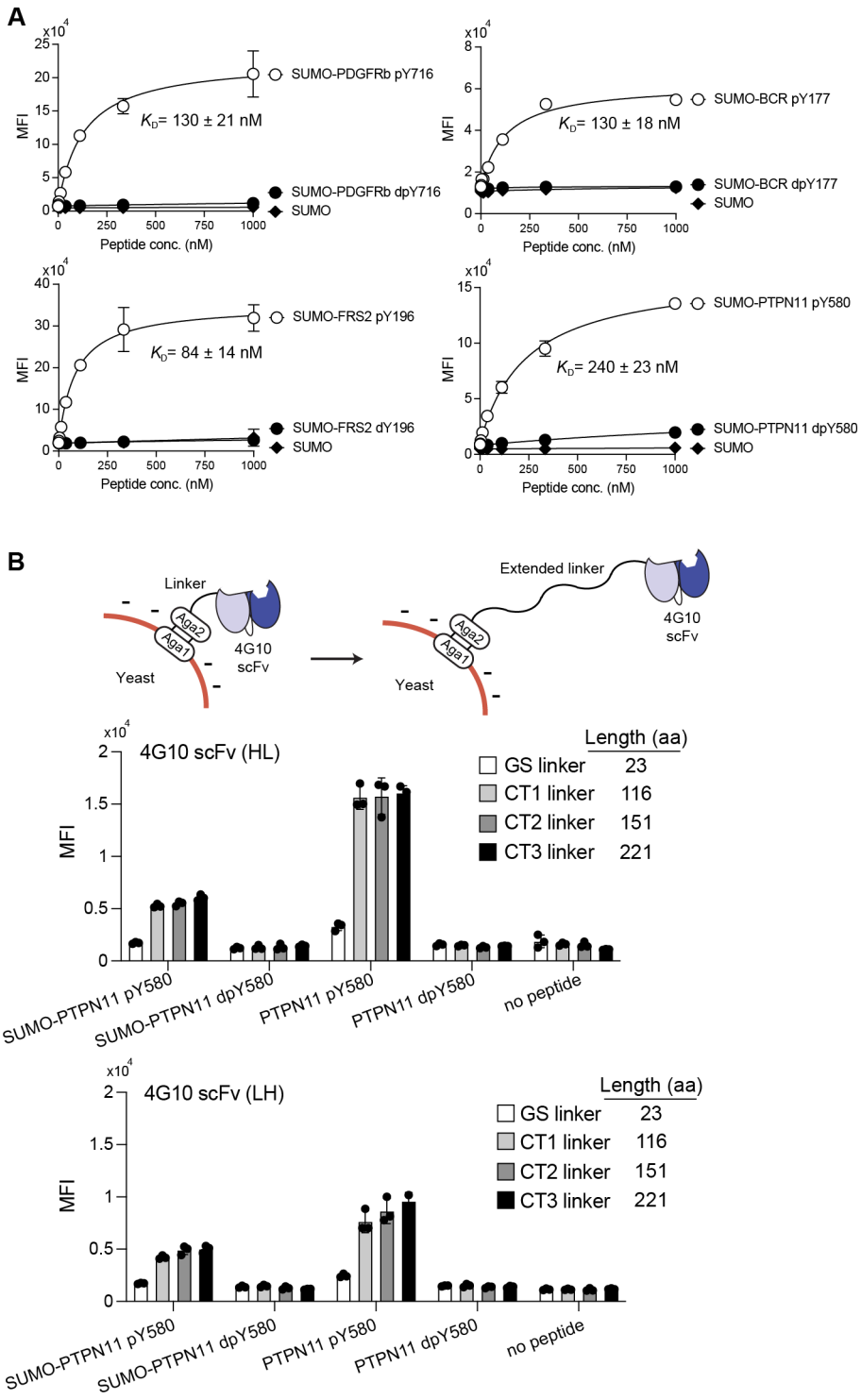


Supplementary Figure S5. Performance comparison of the clasping antibody and the widely-used commercial antibody in native ICeChIP. (A) Scheme of ICeChIP using internal nucleosome standards to calibrate native ChIP. (B) Cartoon representation of the most relevant barcoded nucleosomes. (C) Scatter plots of input and IP reads for each member captured for the indicated barcoded nucleosome standards (symmetric H3K4me3, symmetric H3K27me3, symmetric cis-H3K4me3/H3K27me3 and asymmetric trans-H3K4me3/H3K27me3). Linear regressions and indicated R^2 statistics for the fitting are displayed in corresponding colors. Left: widely-used H3K27me3 monoclonal antibody (C36B11), Right: 3C2-H2 clasping Fab. (D) Another representative locus on chromosome 3 displaying apparent H3K27me3 histone modification density (HMD) with antibodies.



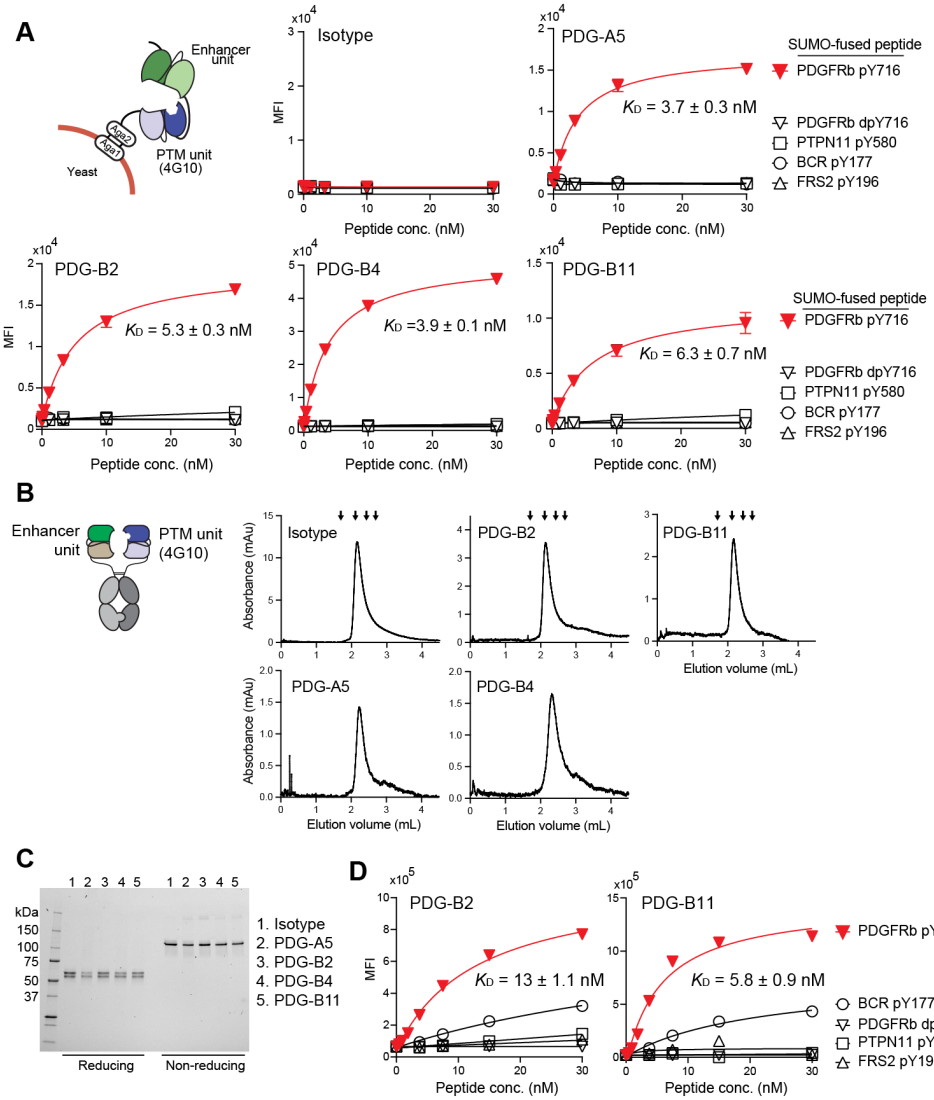
Supplementary Figure S6. Characterization of anti-H3K27me3 long-neck scFv-Fc antibodies and SNAP-ChIP analysis of the commercial recombinant antibody. (A and B) Size-exclusion chromatograms using a Superdex 200 increase 5/150 GL column (A) and SDS-PAGE profiles (B) of purified long-neck scFv-Fc antibodies. The elution positions of standard proteins (with molecular weights of 670, 158, 44, and 17 kDa, respectively) are indicated by arrows in (A). (C) Binding titration curves of long-neck scFv-Fc antibodies against the H3K27me3S28phos peptide with the peptide IP assay. The apparent K_D values calculated from the curve fitting of a 1:1 binding model are shown. Data shown here are from triplicate measurements. Error bars indicate the s.d. (D) The uncropped western blot, related to Fig. 3D. Whole-cell lysate of K562 cells was blotted with the indicated clasping antibodies at three different concentrations (30, 10 and 3.3 nM, respectively). The isotype composes 4-5 (PTM unit) and a non-binding antibody (in replacement of an enhancer unit), and consequently has weak affinity to Kme3. The “secondary only” lanes refer to no primary antibody with anti-mouse Fc-HRP (secondary only 1) or with anti-rabbit IgG-HRP (secondary only 2). The arrow indicates the location corresponding to histone H3. Data shown here are representative of ≥2 equivalent experiments. (E) Native SNAP-ChIP analysis of recombinant anti-H3K27me3 antibody (AB_2793791). The specificity (percentage of off-target immunoprecipitation relative to the on-target, left panel) and enrichment (percentage of

nucleosomes recovered after immunoprecipitation relative to input, right panel) assessed by qPCR are shown. Data shown here are from triplicate measurements. Error bars indicate the s.d.

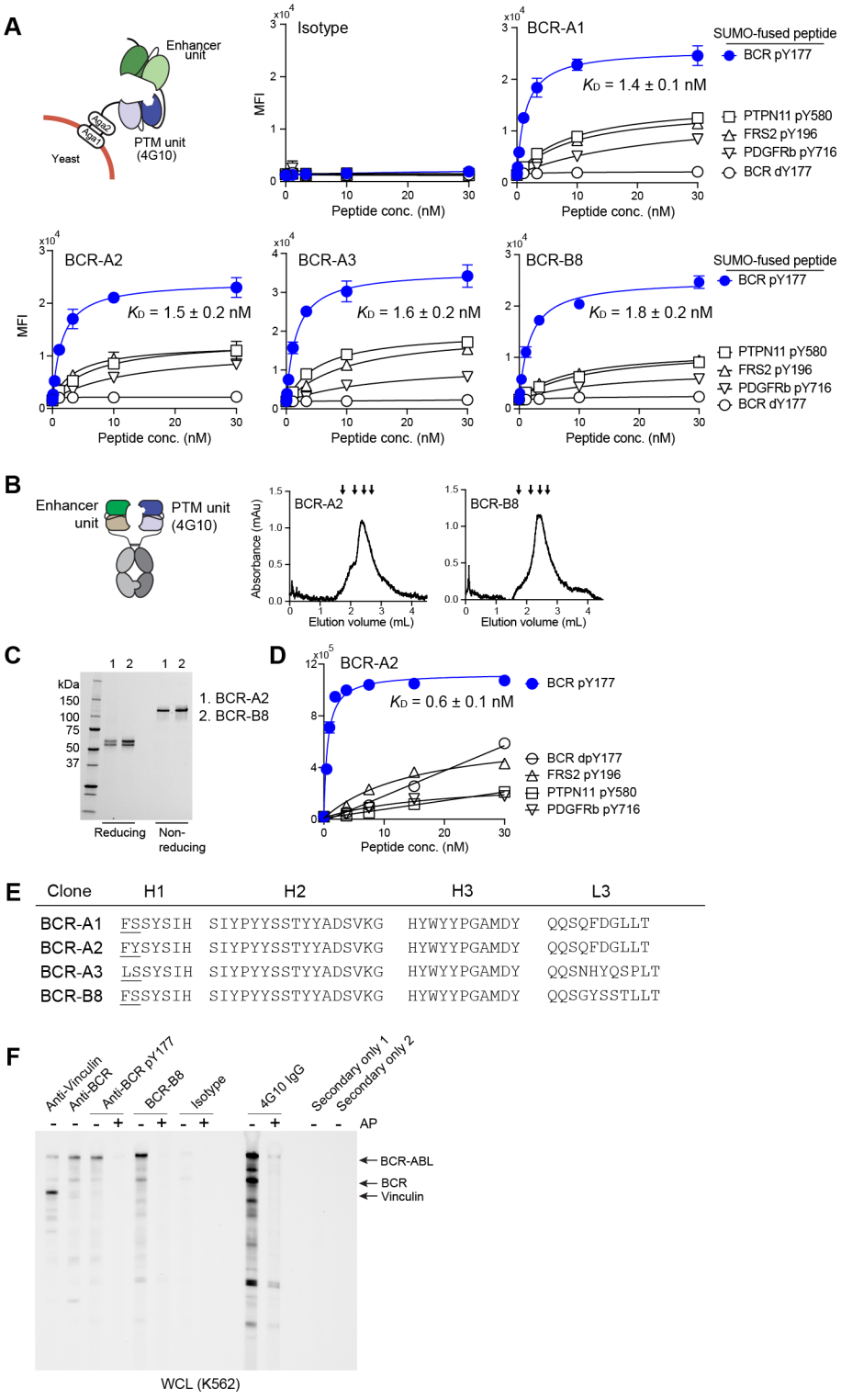


Supplementary Figure S7. Binding characterization of 4G10, anti-pan-pY antibody, in the Fab and yeast-displayed formats. (A) The binding analysis of 4G10 in the Fab format with the peptide IP assay. The SUMO-fused peptides harboring phosphorylated tyrosine were used. The apparent K_D values were calculated from the curve fitting of a 1:1 binding model. (B) The binding analysis of 4G10 scFv in the yeast-display format. The cartoon on the top depicts the extension of the linker between 4G10 scFv and Aga2 protein. The binding of 4G10 scFv in the domain order of

heavy and light variable chains (middle panel) and of light and heavy variable chains (bottom panel) with different linker lengths was tested against indicated peptides at 500 nM concentration. Extending the linker length restored the binding of 4G10 scFv. The abbreviation "dpY" stands for dephosphorylated tyrosine, indicating that the phosphorylation of pY peptides was removed prior to the experiments. Data shown here are from triplicate measurements. Error bars indicate the s.d.


A
Enhancer unit
PTM unit (4G10)
Yeast
Aga2
Aga1
Isotype
PDG-A5
PDG-B2
PDG-B4
PDG-B11
MFI
Peptide conc. (nM)
KD = 3.7 ± 0.3 nM
KD = 5.3 ± 0.3 nM
KD =3.9 ± 0.1 nM
KD = 6.3 ± 0.7 nM
SUMO-fused peptide
PDGFRb pY716
PDGFRb dpY716
PTPN11 pY580
BCR pY177
FRS2 pY196
B
Enhancer unit
PTM unit (4G10)
Isotype
PDG-B2
PDG-B11
PDG-A5
PDG-B4
Absorbance (mAu)
Elution volume (mL)
C
kDa
150
100
75
50
37
1 2 3 4 5
1 2 3 4 5
Reducing
Non-reducing
1. Isotype
2. PDG-A5
3. PDG-B2
4. PDG-B4
5. PDG-B11
D
PDG-B2
PDG-B11
MFI
Peptide conc. (nM)
KD = 13 ± 1.1 nM
KD = 5.8 ± 0.9 nM
PDGFRb pY716
BCR pY177
PDGFRb dpY716
PTPN11 pY580
FRS2 pY196
E
Clone H1 H2 H3 L3
PDG-A5 FSSSSIH SISSSSGSTSYADSVKG YSGYYYSYWYYSWAFDY QQSSSSLIT
PDG-B2 VSSSSIH SISSSSGSTSYADSVKG QYFYYYSSWYWSYALDY QQSSSSLIT
PDG-B4 FSSSSIH SISPSYGSTSYADSVKG YSGYYYSYWYYSWAFDY QQSSSSLIT
PDG-B11 FSSSSIH SISPSYGSTSYADSVKG QYFYYYSSWYWSYALDY QQSHSSLLT
F
Secondary only1
Anti-Vinculin
Anti-PDGFRb
PDG-A5
PDG-B4
4G10 IgG
Isotype
Secondary only2
AP
PDGFRb
Vinculin
WCL (NIH-3T3 treated with PDGF-BB)
WCL (K562)

Supplementary Figure S8. Characterization of clasping antibodies targeting PDGFRb pY716. (A) The binding analysis of clasping antibodies to PDGFRb pY716 in the yeast-display format. (B and C) Size-exclusion chromatograms using a Superdex 200 increase 5/150 GL column (B) and SDS-PAGE profiles (C) of purified long-neck scFv-Fc antibodies. The elution positions of standard proteins (with molecular weights of 670, 158, 44, and 17 kDa, respectively) are indicated by arrows in (B). The 4G10 scFv was used as the PTM unit. (D) Binding titration curves of long-neck scFv-Fc antibodies against the target and off-target peptides with the peptide IP assay. The abbreviation of "dpY" denotes dephosphorylated tyrosine, indicating that the phosphorylation of pY peptides was removed prior to the experiments. The apparent K_D values were calculated from the curve fitting of a 1:1 binding model. Data shown here are from triplicate measurements. Error bars indicate the s.d. (E) CDR sequences of enhancer antibodies for PDGFRb pY716. Residues that are randomized in the antibody library but located outside CDRH1 are indicated by an underscore. (F) The uncropped western blots, related to Figure 4C. The whole cell lysates (WCLs) from NIH 3T3 cells treated with PDGF-BB (left blot) and from K562 cells (right blot) were blotted with indicated clasping antibodies in the long-neck scFv-Fc format or control IgGs. The isotype composes 4G10 (PTM unit) and a non-binding antibody (in place of an enhancer unit), and consequently has weak affinity to pY. The abbreviation "AP" stands for alkaline phosphatase and each lane displaying "+" was treated with AP prior to being blotted with antibodies. The "secondary only" lanes refer to no primary antibody with anti-mouse Fc-HRP (secondary only 1) or with anti-rabbit-IgG HRP (secondary only 2). The arrows indicate the locations corresponding to PDGFRb and vinculin. The data shown are representative of ≥2 equivalent experiments.



Supplementary Figure S9. Characterization of clasping antibodies targeting BCR pY177. (A) The binding analysis of clasping antibodies to BCR pY177 in the yeast-display format. (B and C) Size-exclusion chromatograms using a Superdex 200 increase 5/150 GL column (B) and SDS-PAGE profiles (C) of purified long-neck scFv-Fc antibodies. The elution positions of standard proteins (with molecular weights of 670, 158, 44, and 17 kDa, respectively) are indicated by

arrows in (B). The 4G10 scFv was used as the PTM unit. (D) Binding titration curves of long-neck scFv-Fc antibodies against the target and off-target peptides with the peptide IP assay. The abbreviation of “dpY” denotes dephosphorylated tyrosine, indicating that the phosphorylation of pY peptides was removed prior to the experiments. The apparent K_D values were calculated from the curve fitting of a 1:1 binding model. Data shown here are from triplicate measurements. Error bars indicate the s.d. (E) CDR sequences of enhancer antibodies for BCR pY177. Residues that are randomized in the antibody library but located outside CDRH1 are indicated by an underscore. (F) The uncropped western blots, related to Figure 4F. The whole cell lysate (WCL) from K562 cells was blotted with indicated clasping antibodies in the long-neck scFv-Fc format or control IgGs. The isotype composes 4G10 (PTM unit) and a non-binding antibody (in place of an enhancer unit), and consequently has weak affinity to pY. The abbreviation “AP” stands for alkaline phosphatase and each lane displaying “+” was treated with AP prior to being blotted with antibodies. The “secondary only” lanes refer to no primary antibody with anti-mouse Fc-HRP (secondary only 1) or with anti-rabbit IgG-HRP (secondary only 2). The arrows indicate locations corresponding to BCR-ABL, BCR or vinculin. The data shown are representative of ≥2 equivalent experiments.

SI References

1. T. Hattori *et al.*, Recombinant antibodies to histone post-translational modifications. *Nat Methods* **10**, 992-995 (2013).
2. T. Hattori *et al.*, Antigen clasping by two antigen-binding sites of an exceptionally specific antibody for histone methylation. *Proc Natl Acad Sci U S A* **113**, 2092-2097 (2016).
3. N. Yasui *et al.*, Directed network wiring identifies a key protein interaction in embryonic stem cell differentiation. *Mol Cell* **54**, 1034-1041 (2014).
4. K. R. Miller *et al.*, T cell receptor-like recognition of tumor in vivo by synthetic antibody fragment. *PLoS One* **7**, e43746 (2012).
5. A. Koide, R. N. Gilbreth, K. Esaki, V. Tereshko, S. Koide, High-affinity single-domain binding proteins with a binary-code interface. *Proc Natl Acad Sci U S A* **104**, 6632-6637 (2007).
6. P. S. Lown *et al.*, Extended yeast surface display linkers enhance the enrichment of ligands in direct mammalian cell selections. *Protein Eng Des Sel* **34** (2021).
7. A. Koide, S. Koide, Affinity maturation of single-domain antibodies by yeast surface display. *Methods Mol Biol* **911**, 431-443 (2012).
8. S. Nishikori *et al.*, Broad ranges of affinity and specificity of anti-histone antibodies revealed by a quantitative peptide immunoprecipitation assay. *J Mol Biol* **424**, 391-399 (2012).
9. A. T. Grzybowski, Z. Chen, A. J. Ruthenburg, Calibrating ChIP-Seq with Nucleosomal Internal Standards to Measure Histone Modification Density Genome Wide. *Mol Cell* **58**, 886-899 (2015).
10. R. N. Shah *et al.*, Examining the Roles of H3K4 Methylation States with Systematically Characterized Antibodies. *Mol Cell* **72**, 162-177 e167 (2018).
11. A. T. Grzybowski, R. N. Shah, W. F. Richter, A. J. Ruthenburg, Native internally calibrated chromatin immunoprecipitation for quantitative studies of histone post-translational modifications. *Nat Protoc* **14**, 3275-3302 (2019).