Supplemental Figure Legends

Figure S1. Recombinant Protein Purification, Validation of Knockdown Efficiency and Specificity of siRNAs, related to Figure 1

(**A-D**) Expression and purification of recombinant GST- and His-tagged proteins. Bacterially-expressed GST-Suv39h1 wild type (wt), H320R and H324L mutants (A), His-E2F1 (B), His-Pc2 wt (C) and K191R mutant (D) were analyzed by SDS-PAGE followed by Coomassie blue staining. (**E**) Schematic illustration of synthesized peptides corresponding to Pc2K191me^2 and Pc2K191 antibodies generation. (**F**) Determination of knockdown efficiency and specificity of siRNAs in HeLa cells. Immunoblots showing the knockdown efficiency and specificity of siRNAs targeting indicated proteins at protein level. GAPDH was taken as loading control.

Figure S2. KDM4C-mediated Pc2K191me^2 Instead of H3K9me^2 Demethylation on Growth Control Gene Promoters upon Serum Stimulation, related to Figure 2

(**A**) Expression and purification of histone demethylases in 293T cells. The FLAG-tagged demethylases were purified by 3xFLAG agarose and analyzed by silver staining. (**B**) Analysis of substrate specificity of recombinant FLAG-demethylases purified from 293T cells. Equal amounts of recombinant Histone H3 (dimethyl Lys9) were subjected to histone demethylase assays in the presence of FLAG-demethylases. Changes in histone methylation levels were analyzed by site-specific methyl-lysine antibodies. Immunoblotting with antibodies against histone H3 was used to demonstrate equal loading. (**C**) Expression and purification of His-KDM4C in bacteria. Left panel: A Coomassie blue stained protein gel showing recombinant His-KDM4C (wt and H190G/E192A mutant) purified from bacteria. Right panel: Analysis of substrate specificity of recombinant His-KDM4C (wt and H190G/E192A mutant). Equal amounts of recombinant Histone H3 (dimethyl Lys9) were subjected to histone demethylase assays in the presence of His-KDM4C (wt and H190G/E192A mutant). Changes in histone methylation levels were analyzed by site-specific methyl-lysine antibodies. Immunoblotting with antibodies against histone H3 was used to demonstrate equal loading. (**D**) Determination of knockdown efficiency and specificity of KDM4C siRNAs in HeLa cells at protein level. GAPDH was taken as loading control. (**E**) KDM4C

mediated Pc2K191me^2 instead of H3K9me^2 demethylation on growth control gene promoters. HeLa cells transfected with control or KDM4C siRNAs were serum-starved followed by restimulation and ChIP analyses using Pc2K191me^2 antibodies (top panel) or H3K9me^2 antibodies (bottom panel) were performed on indicated regions.

Figure S3. Validation of Knockdown Efficiency and Specificity of Pc2 siRNA/shRNA, related to Figure 3

(**A**) Determination of knockdown efficiency and specificity of siRNA targeting Pc2 in HeLa cells at protein level. (**B)** Determination of knockdown efficiency of shRNA targeting Pc2 in IMR-90 cells. GAPDH was taken as loading control.

Figure S4. Relocation of E2F1-regulated Genes from PcG Bodies to Interchromatin Granules and Serum-induced Colocalization of Growth Control Genes with Unmethylated Pc2, related to Figure 4

(**A**-**C**) E2F-regulated growth control genes including *MCM3* (A), *PCNA* (B) and *MSH2* (C) relocate between PcG bodies and interchromatin granules upon serum treatment. HeLa cells were serum-starved followed by restimulation and Immuno-FISH analyses were performed using antibodies and probes as indicated. (**D**-**G**) Serum-induced colocalization of E2F1 target gene loci with unmethylated Pc2. HeLa cells were serum-starved followed by restimulation and Immuno-FISH analyses were performed using Pc2K191 antibody and probes as indicated. (**H**) HeLa cells were serum-starved followed by restimulation and Immuno-FISH analyses were performed using Ring1A or SC35 antibodies and probes targeting *BCR* locus. (**I**) Determination of knockdown efficiency and specificity of Bmi1, Ring1A and PHC1 siRNAs in HeLa cells at protein level. GAPDH was taken as loading control. (**J**) Effect of knockdown Pc2, Bmi1 and PHC1 on PcG bodies structure. HeLa cells transfected with indicated siRNA were subjected to immunostaining with Ring1A antibodies. For A-G, Left panel: representative images; right panel: statistics analysis. Mean$\pm$SEM, *$p<0.05$, **$p<0.01$ and ***$p<0.001$.

Figure S5. CLIP Sequencing, *In vitro* Transcription of Biotinylated RNAs, MODified Histone Peptide Array® Image, **Validation of Knockdown Efficiency of**

***TUG1* and *NEAT2* and Nuclear Localization of *TUG1*and *NEAT2*, related to Figure 5**

(**A** and **B**) Sequencing results of RNAs associated with methylated and unmethylated Pc2. 94 individual colonies were subjected to automatic sequencing and summary of the cloned sequences associated methylated (A) or unmethylated (B) Pc2 is shown. (**C** and **D**) Denaturing agarose gel stained with EtBr showing *in vitro* transcribed *TUG1* (C) and *NEAT2* (D) fragments. Left panel: sense RNA transcripts; right panel: antisense RNA transcripts. (**E**) EMSA competition assay demonstrating specificity of *TUG1* and *NEAT2* probes. *In vitro* methylated or unmethylated His-Pc2 was incubated with ^{32}P-labeled *TUG1* or *NEAT2* probes, respectively, and where indicated, a molar excess (50 or 200×) of unlabeled competitors (cold) were added in the reactions. (**F-H**) MODified Histone Peptide Array® images corresponding to **Figures 5D-5F**. MODified Histone Peptide Array® was incubated with Recombinant His-Pc2 chromodomain in the presence of yeast tRNA (F), *in vitro* transcribed *TUG1* (117-3390) (G), or *NEAT2* (2281-5611) (H) RNA fragments and subjected to immunoblotting with indicated antibodies. The Myc tag antibody was added to detect Myc tagged peptides on position P21 as positive control. (**I** and **J**) No interaction between *TUG1* (117-3390), or *NEAT2* (2281-5611) with histone marks. MODified Histone Peptide Array® was incubated with *in vitro* transcribed *TUG1* (117-3390) (I), or *NEAT2* (2281-5611) (J) RNA fragments and subjected to immunoblotting with Streptavidin-HRP. The binding specificity calculated by Array Analyses Software based on two images was shown. (**K**) qRT-PCR results showing the mRNA level of *TUG1* (left panel) and *NEAT2* (right panel) in HeLa cells transfected with corresponding siRNAs against *TUG1* or *NEAT2*. (**L**) Effect of *TUG1* knockdown on PcG bodies structure. HeLa cells transfected with indicated siRNA were serum-starved followed by restimulation and immunostaining was performed using Ring1A antibodies. (**M**) *TUG1* and *NEAT2* ncRNAs preserve nuclear structure upon serum stimulation. Serum-starved and stimulated HeLa cells were subjected to Stellaris™ RNA FISH probes targeting *TUG1* (left panel) and *NEAT2* (right panel).

Figure S6. Biochemical Characterizations of E2F1 SUMOylation, related to Figure 6

(**A**) Validation of denaturing conditions for immunoprecipitation. HeLa cell lysates were subjected to immunoprecipitation in denaturing condition as indicated. The dissociation was measured by the absence of Rb, a known E2F-1-associated protein in the E2F1 immunoprecipitates. (**B**) SUMO1 specific conjugation of E2F1. HeLa cells were transfected with indicated plasmids and the cell lysates were immunoprecipitated with E2F1 antibodies under denaturing conditions followed by immunoblotting with E2F1 (left panel), Myc tag (middle panel) and HA tag (right panel) antibodies. (**C**) Biochemical characterization of E2F4 and E2F6 SUMOylation. HeLa cells were transfected with indicated plasmids and the cell lysates were immunoprecipitated with E2F4 antibodies (upper panel) or E2F6 antibodies (bottom panel) under denaturing conditions followed by immunoblotting with E2F4 or E2F6 (left panel), Myc tag (middle panel) or HA tag (right panel) antibodies. (**D**) Determination of knockdown efficiency and specificity of Ubc9, PIAS3 and Pc2 siRNAs in HeLa cells at protein level. GAPDH was taken as loading control. (**E**) Overexpression of Ubc9 and Pc2 enhances E2F1 SUMOylation. HeLa cells were co-transfected with Myc-SUMO1 and plasmids as indicated. The cell lysates were immunoprecipitated with E2F1 antibodies under denaturing conditions and the immunoprecipitates were subjected to immunoblotting with E2F1 (left panel) and Myc tag (right panel) antibodies. (**F**) *In vitro* SUMOylation of E2F1 at K266. Bacterially-expressed His-E2F1 (wt or K266R mutant) were incubated with recombinant E1, E2, His-Pc2 and SUMO1. The reaction products were separated by SDS-PAGE and subjected to Coomassie blue staining (left panel), immunoblotting with E2F1 (middle panel) or SUMO1 (right panel) antibodies. (**G**) E2F1 SUMOylation is required for growth control gene expression. HeLa cells transfected with blank vector, E2F1wt or K266R mutant were serum-starved followed by restimulation and relative mRNA levels of indicated genes were determined by qRT-PCR. (**H**) No effects of E2F1 K266R mutation on E2F1 stability and Rb interaction. HeLa cells transfected with FLAG-E2F1 (wt or K266R mutant) were serum-starved followed by restimulation and the cell lysates were immunoprecipitated with FLAG antibodies followed by immunoblotting with E2F1 (upper panel) and Rb (middle panel) antibodies. Bottom panel shows the input proteins used in immunoprecipitation assays. (**I**) Overexpression of Pc2 K191R mutant does not change the expression levels of Ubc9 and SUMO activating enzyme E1 and E2. HeLa

cells were transfected with indicated plasmids and the cell lysates were immunoblotted with indicated antibodies. Mean±SEM, *p<0.05 and **p<0.01.

Figure S7. CDC7AL-mediated Histone H2B Ubiquitination, related to Figure 7

(**A**) MS/MS results showing the identification of CDCA7L. (**B**) CDCA7L interacts with SUMOylated E2F1 *in vivo*. HeLa cells transfected with indicated plasmids were serum-starved followed by restimulation and the cell lysates were immunoprecipitated with FLAG antibody followed by immunoblotting with E2F1 or CDCA7L antibodies. (**C**) Determination of knockdown efficiency and specificity of CDCA7L shRNAs in HeLa cells at protein level. GAPDH was taken as loading control. (**D**) CDCA7L serves as an ubiquitin E3 ligase for histone H2B. The potential ubiquitin-conjugated proteins were purified by Nickel bead under denaturing conditions in HeLa cells co-transfected with FLAG-CDCA7L and His-ubiquitin and followed by immunoblotting with H2B (left panel) or H2A (right panel) antibodies. (**E**) CDCA7L ubiquitinates histone H2B at lysine 120 *in vivo*. HeLa cells were transfected with indicated plasmids and the cell lysates were subjected to immunoblotting with H2B or UbH2B antibodies. (**F**) CDCA7L ubiquitinates histone H2B *in vitro*. Bacterially-expressed His-CDCA7L (wt or C353/356S mutant) was incubated with recombinant His-Ubc6, UBE1, ubiquitin and Histone H2B. The reactions were separated by SDS-PAGE followed by Coomassie blue staining (left panel) and immunoblotting with H2B (middle panel) or UbH2B (right panel) antibodies.

Extended Experimental Procedures

Cloning Procedures

The SUMO1, SUMO2, SUMO3 expression vectors have been previously described (Ghisletti et al., 2007; Pascual et al., 2005). Full-length Suv39h1, Pc2, E2F1, KDM3A, KDM4A, KDM4B, KDM4C, KDM4D, and PHF8 were PCR-amplified from cDNAs generated from HeLa cells by using KOD Hot Start Master mix (Novagen) and cloned into p3XFLAG-CMV™-10 (Sigma) vector. Expression vectors encoding full-length human Ubc9, CDCA7L, and Ubiquitin were purchased from OriGene. Ubiquitin sequence was subcloned into pCDNA™ 3.1-His (Invitrogen) to construct His-Ubiquitin expression vector. Bacterial expression vectors for Pc2, E2F1, KDM4C, and CDCA7L

were constructed by subcloning the gene sequences into pET-28a backbone (Novagen). For GST-Suv39h1 construct, Suv39h1 sequence was subcloned into pGEX-JDK backbone (GE Healthcare Life Sciences). Human *TUG1* and *NEAT2* were amplified from cDNAs generated from HeLa cells and cloned into pSTBlue-1 vector (Novagen) for *in vitro* transcription assay. All mutants were generated by using QuikChange™ Lightning Site-Directed Mutagenesis Kit (Agilent Technologies). Oligonucleotides for shRNA targeting Pc2-603 (AAA AGC GGC AAG TAC TAC TAC CAG CTC AAT TGG ATC CAA TTG AGC TGG TAG TAG TAC TTG CCG C) and Pc2-2598 (AAA ACC AAC TAT TAC CTG CTT GAA TGT AAT TGG ATC CAA TTA CAT TCA AGC AGG TAA TAG TTG G) were subcloned into pLV-RNAi according to manufacturer's instructions (Biosettia).

RNA Interference

Commercially available ON-TARGET*plus* SMARTpool® siRNAs targeting Suv39h1 (L-009604), Ubc9 (L-004910), PIAS3 (L-004164), Suv39h2 (L-008512), G9a (L-006937), EuHMTase (L-007065), ESET (L-020070), KDM4C (L-004293), Pc2 (L-008356), Bmi1 (L-005230) and PHC1 (L-011850) from Dharmacon were used in this study. HuSH 29mer shRNA constructs against CDCA7L were purchase from OriGene. *TUG1* and *NEAT2* custom ON-TARGET*plus* SMARTpool® siRNAs were synthesized by Dharmacon with the following sequences: *TUG1* (GGA TAT AGC CAG AGA ACA A, GTG CAG AAG CCC AGA GTA A, GAA TAA GCC CTA TGG ATT A, CCA GAA GAG TTA AGA ATC A) and *NEAT2* (GAT CAG GAT TTG AGC GGA A, GGA AGG AGC GCT AAC GAT T, AAG CAG AAT AAA AGC GAA A, AAG ATA TTG CTT AGC GTT A). siRNA transfections were done with 75 nM of siRNA and Lipofectamine™ 2000 (Invitrogen) following manufacturer's instructions.

Antibodies

The following antibodies were used for immunoprecipitation and immunoblotting: E2F1 (C-20), E2F1 (KH95), GAPDH (6C5), Ubiquitin (P4D1), SUMO-1 (D-11), G9a (C-15), EHMT1 (T-22), ESET (A-1), Suv39h2 (Z-23), CtBP (H-440), CtBP (E-12), Ubc9 (H-81), SUMO-2/3 (N-18), E2F4 (C-20), E2F4 (D-3), E2F6 (H-50), E2F6 (TFE61), and 14-

3-3β (A-6) from Santa Cruz Biotechnology. Pan Pc2, PHC1, KDM4C, FLAG® M2, and CDCA7L from Sigma-Aldrich Prestige Antibodies®. Suv39h1, Rb, His tag (27E8), HA tag (6E2), PIAS3, Histone H2A and Ring1A from Cell Signaling. Myc tag (4A6), Bmi1 (clone F6), Histone H2B, and Ubiquityl-Histone H2B (clone 56) from Millipore. H3K9me^2 and Histone H3 from Active Motif. SAE1 and SAE2 from Abcam.

The following antibodies were used for ChIP: Pan Pc2 (T-20) and SUMO-1 (D-11) from Santa Cruz Technology, FLAG® M2 and CDCA7L from Sigma-Aldrich. H3K9me^2 from Abcam. ChIPAb$^+$ Histone H2B and Ubiquityl-Histone H2B (clone 56) from Millipore.

The following antibodies were used for immunostaining: Bmi1 (Prestige Antibodies®) from Sigma-Aldrich, Ring1A from Abcam and SC35 was previously described (Fu and Maniatis, 1990).

Lentiviral-based Gene Transduction

Production of Lenti-shRNA viral stocks was performed in 293LTV cell line according to manufacturer's instruction (Cell Biolabs). The lentivirus was further purified and concentrated by ViraBind™ Lentivirus Concentration and Purification Kit (Cell Biolabs). IMR-90 fibroblasts were transduced by using ViraDuctin™ Lentivirus Transduction Kit from Cell Biolabs and purified with 1.2 ug/ml Puromycin.

Cell Lysis, Protein Immunoprecipitation, and Immunoblotting

Cells were homogenized in 1xRIPA buffer with protease and SUMO proteases inhibitor N-ethylmaleimide (NEM) (Calbiochem®). Lysates were cleared by centrifugation at 13,000 rpm for 15 min at 4°C. Supernatants were analyzed for immunoblotting or for immunoprecipitation with the indicated antibodies. For immunoprecipitation under denaturing conditions, cell lysate was first heated in the presence of 1% SDS and 5 mM DTT to disassociate protein-protein interactions before being subjected to immunoprecipitation. The disassociation was measured by the absence of Rb, a known E2F1-associated protein in the E2F1 immunoprecipitates (**Figure S6A**).

RNA Gel Mobility Shift Assay

The gel mobility shift assay was performed with bacterial recombinant full length Pc2 (as described in **Protein Recombination and Purification** section) and synthesized RNA oligonucleotides using Gel Shift Assay System (Promega) followed by fractionation on 6% DNA retardation gels (Invitrogen). The gels were supported on GelBond® PAG film (Lonza) and exposed to X-ray film at –70 °C for overnight.

RNA Pulldown Assay

Biotin-labeled *TUG1* RNA fragments (117-3390, 3302-4932, and 4771-6986) and *NEAT2* RNA fragments (39-2382, 2281-5611, and 5458-8662) were *in vitro* transcribed with the Biotin RNA Labeling Mix (Roche) and T7 or SP6 RNA polymerase (Promega) and purified with RNeasy® Mini Kit (QIAGEN). RNA electrophoresis was performed using NorthernMax® kit according to manufacturer's manual (Ambion) to demonstrate that all the RNAs are transcribed at the right size. 50 μg of each *TUG1* and *NEAT2* RNA fragments were mixed and incubated with HeLa nuclear extract. To isolate biotinylated RNA bound proteins, 60 μl washed BcMag™ Monomer Avidin Magnetic beads (Bioclone) were added to each binding reaction and further incubated at room temperature for 1 hour. Beads were washed one time with RNA structure buffer (Tsai et al., 2010), two times with PNK buffer (20 mM Tris-HCl, pH 7.4; 10 mM $MgCl_2$; 0.2% Tween-20), four times with high-salt wash buffer (50 mM Tris-HCl, pH 7.4; 1 M NaCl; 1 mM EDTA; 1% NP-40; 0.5% sodium deoxycholate; 0.1% SDS), two times with PBS and bound RNA-protein complexes were eluted by 2 mM biotin in PBS. The elutants were digested using immobilized trypsin (Promega) and subjected to MS analysis at UCSD Biomolecular and Proteomics Mass Spectrometry Facility.

Modified Histone Peptide Array

The interaction between recombinant proteins or *in vitro* transcribed RNA with Histone was analyzed by MODified™ Histone Peptide Array (Active Motif) according to manufacturer's instruction. The specificity of interactions was quantified by Array Analyses Software (Active Motif).

Protein Recombination and Purification

Recombinant His-E2F1 and Pc2 proteins were expressed in *E.coli* strain BL21-CodonPlus® (DE3)-RIPL (Agilent Technologies) and purified using His60 Ni Superflow™ Resin (Clontech). GST-Suv39h1 proteins were purified using GST-Tag Purification Resins (Clontech) from induced *E.coli* strain Rosetta-gami™ (Novagen) grown for 18 hours at room temperature in SOB medium. Recombinant Histone H3 (dimethyl Lys9) was purchased from Active Motif**.**

***In Vitro* Methylation Assay**

Purified recombinant proteins were incubated (1 hour, 30 °C) with 1 μg of recombinant GST-Suv39h1 in 30 μl of methylation buffer (50 mM HEPES [pH 8.0], 0.01% (v/v) NP-40, 10 mM NaCl, 1 mM DTT and 1 mM PMSF) supplemented with 2 μl of *S*-adenosyl-L-[methyl-[^{3}H]methionine ([^{3}H]-SAM, Perkin Elmer; for radioactive methylation) or 20 nmol of *S*-adenosyl-L-methionine sulfate *p*-toluenesulfonate (SAMe-PTS, Sigma-Aldrich; for nonradioactive methylation). To recover the methylated His-tagged protein, PureProteome™ Nickel magnetic beads (Millipore) were added into reactions and the bound proteins were washed and eluted by His-tag elution buffer (50 mM Tris-HCl [pH 8.0], 150 mM NaCl, 300 mM imidazole). The eluted proteins were further dialyzed against the appropriate buffers for downstream assays. Otherwise, SDS loading buffer was added to half of each methylation reactions and boiled followed by separation on a 4-12% SDS-PAGE gel. The resulting protein bands were visualized by Coomassie blue staining, immunoblotting or autoradiography using EN^{3}HANCE™ spray (Pelkin Elmer).

***In Vitro* SUMOylation Assay**

The *in vitro* SUMOylation assay was performed using SUMOlink™ SUMO-1 kit from Active Motif. The bacterially-expressed His-E2F1 and His-Pc2 were used in the assay as indicated. The assays were subjected to SDS-PAGE/Coomassie Blue staining and immunoblotting with E2F1 or SUMO1 antibodies.

***In Vivo* Ubiquitination Assay**

HeLa cells in 10-cm plates were transfected with combinations of 5 μg of His-ubiquitin expression plasmid and 1 to 5 μg of FLAG-CDCA7L expression plasmids. 48 hours after

transfection, cells from each plate were collected into two aliquots. One aliquot (10%) was used for conventional immunoblotting to confirm expression and inputs. The remaining cells (90%) were used for purification of His-tagged proteins by PureProteome™ Nickel magnetic beads (Millipore). The cell pellet was lysed in buffer A (6 M Guanidine-HCl, 0.1 M Na_2HPO_4/NaH_2PO_4, 0.01 M Tris-HCl [pH 8.0], 5 mM imidazole, 10 mM β-mercaptoethanol) and incubated with Nickel magnetic beads for 4 hours at room temperature. The beads were washed with buffer A, buffer B (8 M urea, 0.1 M Na_2PO_4/NaH_2PO_4, 0.01 M Tris-HCl [pH 8.0], 10 mM β-mercaptoethanol), and buffer C (8 M urea, 0.1 M Na_2PO_4/NaH_2PO_4, 0.01 M Tris-HCl [pH 6.3], 10 mM β-mercaptoethanol), and bound proteins were eluted with buffer D (200 mM imidazole, 0.15 M Tris-HCl [pH 6.7], 30% glycerol, 0.72 M β-mercaptoethanol, 5% SDS). The eluted proteins were analyzed by immunoblotting for the presence of ubiquitinated target proteins with antibodies as indicated.

***In Vitro* Ubiquitination Assay**

The bacterially-expressed His-CDCA7L proteins were incubated with commercially available Histone H2B (Active Motif), Ubiquitin (BostonBiochem®), UBE1 (BostonBiochem®) and UbcH6 (BostonBiochem®) in reaction buffer (final volume, 30 µl) containing 50 mM Tris-HCl [pH 7.4], 5 mM $MgCl_2$, 2 mM NaF, 10 nM okadaic acid, 2 mM ATP, 0.6 mM DTT. Reactions were incubated at 37°C for 1 hour, terminated by boiling for 5 min with SDS sample buffer containing 0.1 M DTT, and resolved by SDS-PAGE, followed by Coomassie blue staining and immunoblotting with H2B or Ubiquityl H2B antibodies.

***In Vitro* Histone Demethylase Assay**

Demethylase assay was performed as described (Kokura and Fang, 2009). Briefly, FLAG–KDMs purified from 293T cells or recombinant KDM4C was incubated with histone or methylated Pc2 as appropriately indicated at 37 °C for 2–3 hours in histone demethylation buffer: 50 mM HEPES-KOH [pH 7.5], 70 µM $Fe(NH4)_2(SO4)_2$, 1 mM α-ketoglutarate, and 2 mM ascorbate. Substrate methylation levels were analyzed by immunoblotting with specific antibodies or autoradiography using EN^{3}HANCE™ spray

(Pelkin Elmer).

Mass Spectrometric Analysis

Gel-resolved proteins or proteins pulled down by biotinylated *TUG1* and *NEAT2* were digested with immobilized trypsin (Promega), batch purified on a reversed-phase ZipTip® (Millipore), and resulting peptide pools were then subjected to mass spectrometric analysis at UCSD Biomolecular and Proteomics Mass Spectrometry Facility. All experiments for MS were performed as two biological repeats and data were analyzed using ProteinPilot™ software (Applied Biosystems).

RNA isolation and qRT–PCR

Total RNA was isolated from HeLa cells using RNeasy Mini Kit (Qiagen) following the manufacturer's protocol. First-strand cDNA synthesis from total RNA was carried out using SuperScript® III (Invitrogen). Resulting cDNA was then analyzed by qPCR using Stratagene Mx3000 machine. Primers are specific for genes tested and their sequences are listed in **Oligonucleotide Primers and FISH Probes** section. All qRT–PCRs were repeated at least three times and representative results were shown.

Oligonucleotide Primers and FISH Probes

For ChIP qPCR: *HoxA2* promoter (Forward, CCA GCG AGG CTT TAT TTA CAC TTT; Reverse, CAT TCA CAC ACA CAC ACA CAC ACT), *GAPDH* Exon8 (Forward, CCA TCA CTG CCA CCC AGA AG; Reverse, AGC TTC CCG TTC AGC TCA GG), *RBL1* promoter (Forward, CAG CGT GGG GCT TGT CCT CG; Reverse, AGC GGA GGC AGA CGG TGG AT), *MCM3* promoter (Forward, CTG CGG CAC ACG GTC TGG AG; Reverse, GGA CGA TGT GGA GCT GCG GG), *PCNA* promoter (Forward, GAT GGC CCA CGC CAG CCA AT; Reverse, GCA GCT CAC CCT GCG GTC TG), *MSH2* promoter (Forward, GGT CGG CTT CGT GCG CTT CT; Reverse, TCA CCT GCC GGC CCC ATG TA), *CCNE1* promoter (Forward, GGG GCG CAT ATG GAA GGG GC; Reverse, CTG AGT CCT GGG CGG GGA CA), *CDC25A* promoter (Forward, GTT CTC CCA CCC GCT TGC CC; Reverse, TGG CCC CAC TGA GCC GCT AT), *CCNB1* promoter (Forward, TTT GAC ACT TCT GAG ACT GTG GCC G;

Reverse, CAA GGA CCT ACA CCC AGC AGA AAC C), *DHFR* promoter (Forward, GGG CTG CCA TCC TTG CCC TG; Reverse, CTG GTC GGC TGC ACC TGT GG), *POLA1* promoter (Forward, TGC TCT CCT CCG GCT TCC CC; Reverse, CGC GCC AAC CAG CCC AAA AC), *CDC6* promoter (Forward, CCC CGT TCA GTG GTC GTG GC; Reverse, GCC ACA GCG TTC CCA CCT CC), ORC1 promoter (Forward, TCG AGA GCT CCG GGG GAT GC; Reverse, ACG AAG GGA CGG TCT GGG GG), *H2A* promoter (Forward, TGA GGA CAA GCT GGG TTA TGC CCC TA; Reverse, GCA CTC GTC CTA CAG GAA ATT GGA G), and *Actin* promoter (Forward, TCG AGC CAT AAA AGG CAA CTT TC; Reverse, AAA CTC TCC CTC CTC CTC TTC C).

For FISH: *MCM3* (RP11-349G11, Chr6: 52,211,229-52,372,786 and RP11-318H5, Chr6: 52,236,770-52,257,541), *RBL1* (RP11-332A4, Chr20: 35,073,121-35,152,344), *MSH2* (RP11-436K12, Chr2: 47,555,641-47,694,791), and *PCNA* (RP11-686P6, Chr20: 4,962,138-5,153,894).

For gel mobility shift assay: *TUG1* (ACA CAC AAU AAA UUU GGA CCA GGG GAU UUU UUA GUU AUU AAU GCU); *NEAT2* (CAG UAU GAU GGC CUA GAU GCA GAG AAA ACA GCU CCU UGG UGA AUU GAU AA).

For molecular beacon: *TUG1* (5'-/56-FAM/mCmGmCmGmAmUmCmCmAmGmUmGmAmCmCmAmUmGmAmCmCmUmCmUmAmGmCmGmAmUmCmGmCmG/3BHQ_1/-3')

For gene expression qPCR: *MCM3* (Forward, TCA AGC CTG TCC TGA CAC AG; Reverse, CAG GTC CAC AGT CTT GCT CA), *PCNA* (Forward, GAA GCA CCA AAC CAG GAG AA; Reverse, TCA CTC CGT CTT TTG CAC AG), *MSH2* (Forward, GCT GGA AAT AAG GCA TCC AAG; Reverse, CCA CAT ACC CAA CTC CAA CC), *CDC25A* (Forward, GAG ATC GCC TGG GTA ATG AA; Reverse, TGC GGA ACT TCT TCA GGT CT), *CCNE1* (Forward, ATC CTC CAA AGT TGC ACC AG; Reverse, AGG GGA CTT AAA CGC CAC TT), *RBL1* (Forward, ATG GAT GCT CCA CCA CTC TC; Reverse, GAG CGC TTC TTG GTG TAA GG), *HSP70* (Forward, AAA GGT CGT CTG AGC AAG GA; Reverse, TTT CTC GTC TTC CAC CGT CT), *TK* (Forward, GAC ATC AGC CTG CTT CTT CC; Reverse, AGG TAG GAA GGG CTT TGA GC), *Suv39h1* (Forward, GCT ATG ACT GCC CAA ATC GT; Reverse, ACA CGT CCT CCA CGT AGT C), *E2F1* (Forward, AGG GTT TCC AGA GAT GCT CA;

Reverse, TGC ATG CAC ATA CAC ACC AC), *TUG1* (Forward, AGT GAA TTA TGT CCT GTG CCT; Reverse, GAT GGG TGA ATG CCT CCT G), *NEAT2* (Forward, TGG GGG AGT TTC GTA CTG AG; Reverse, TCT CCA GGA CTT GGC AGT CT), and *Actin* (Forward, GCT CGT CGT CGA CAA CGG CTC; Reverse, CAA ACA TGA TCT GGG TCA TCT).

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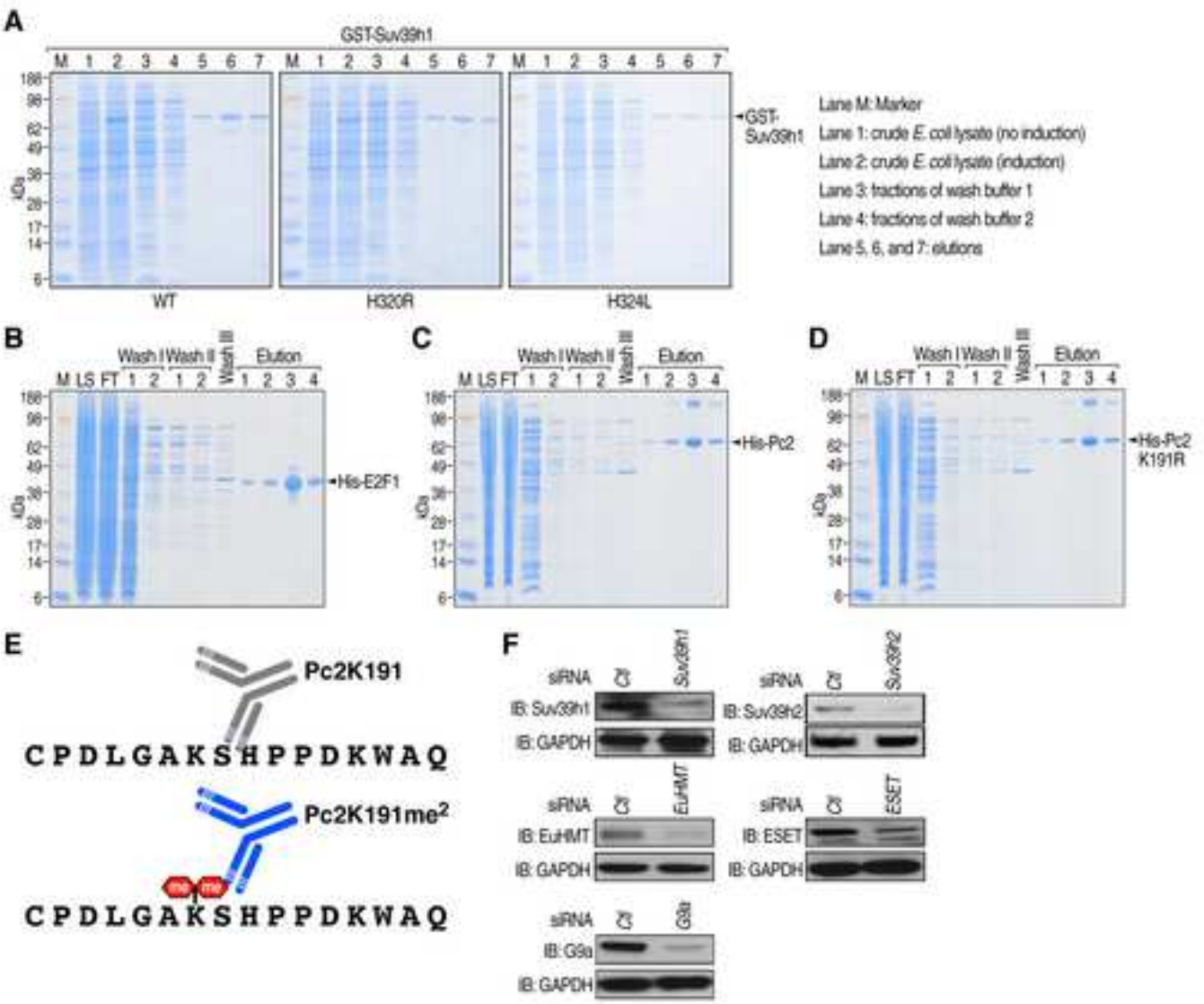

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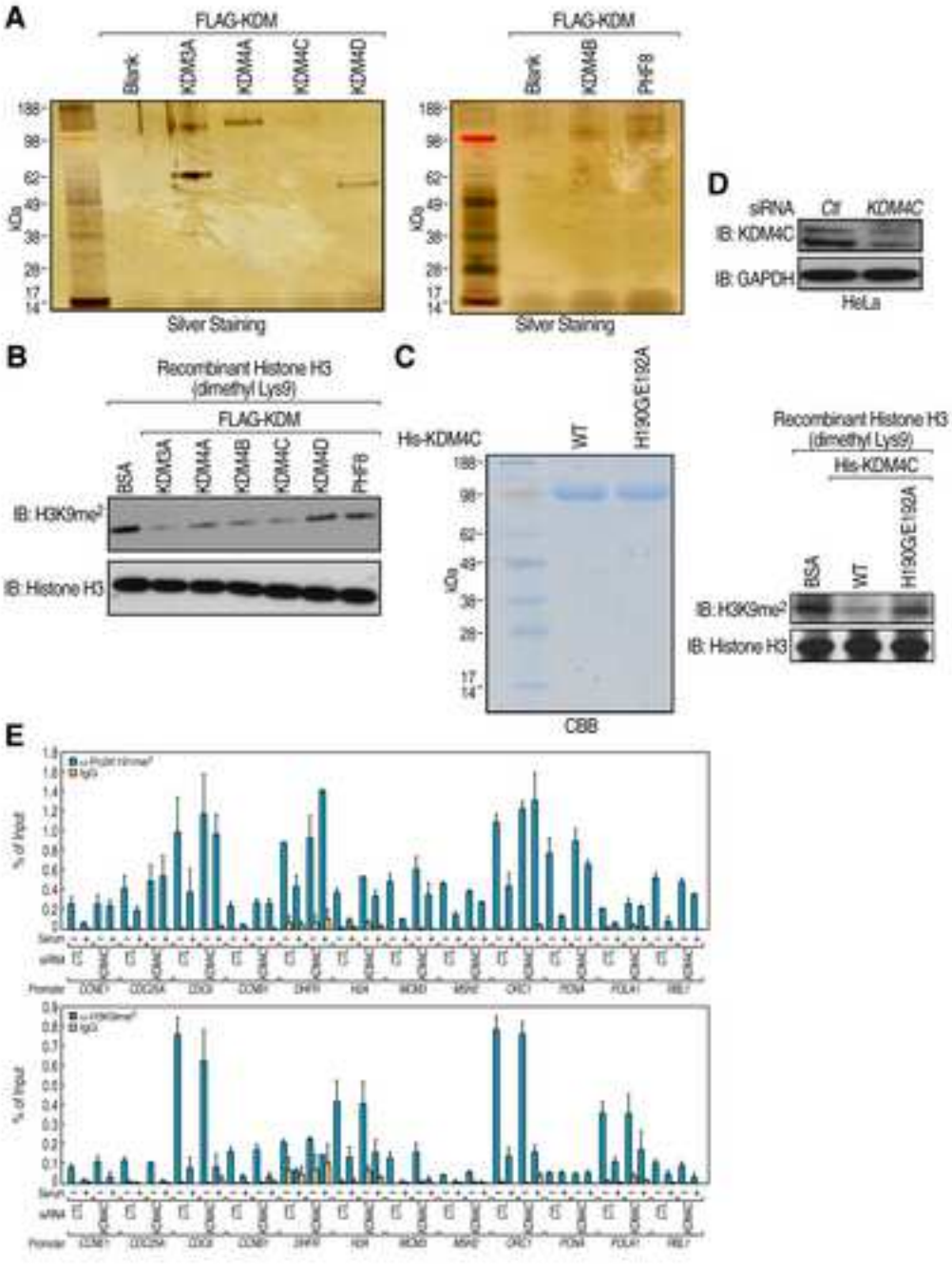

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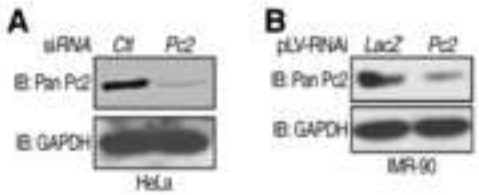

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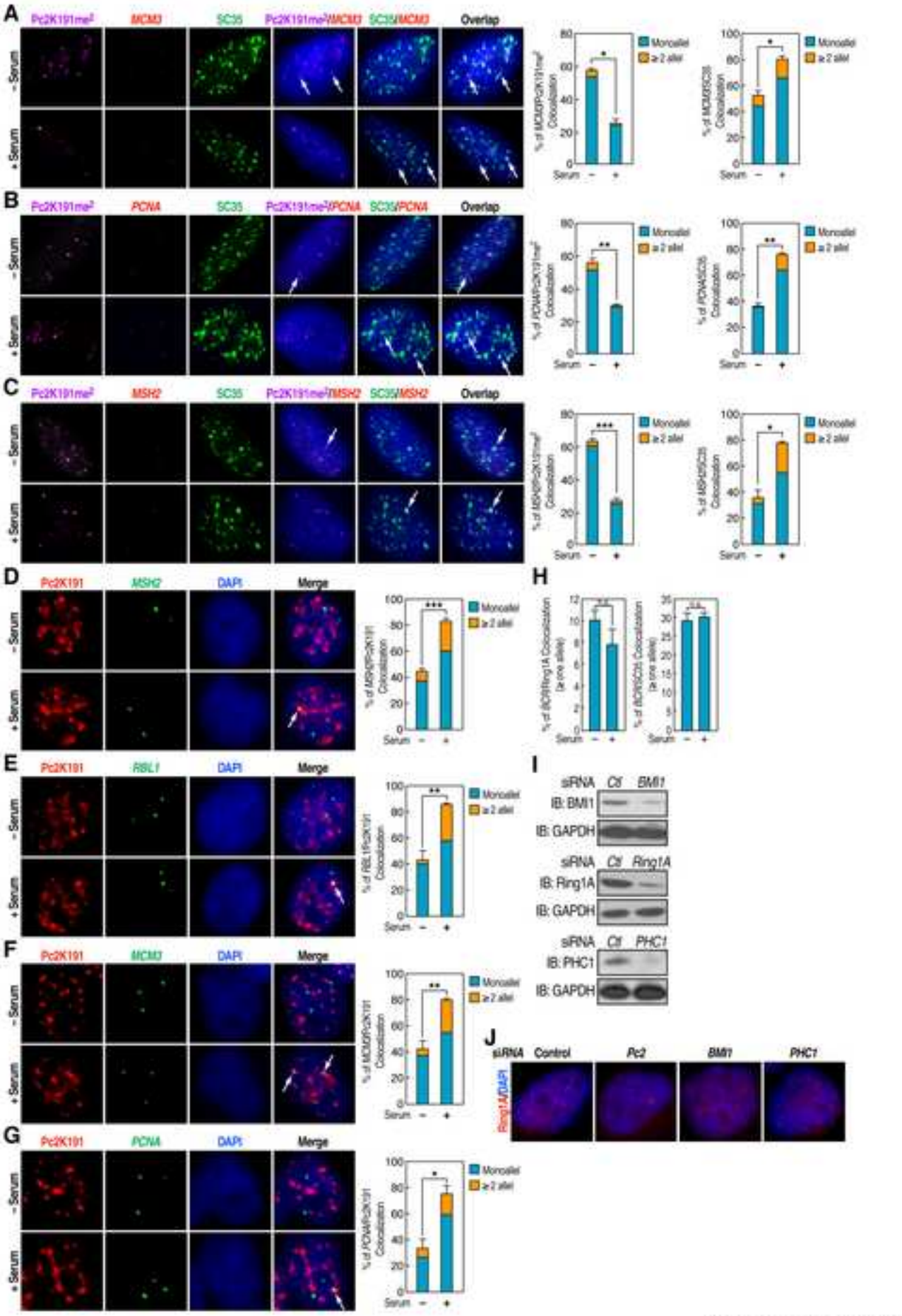

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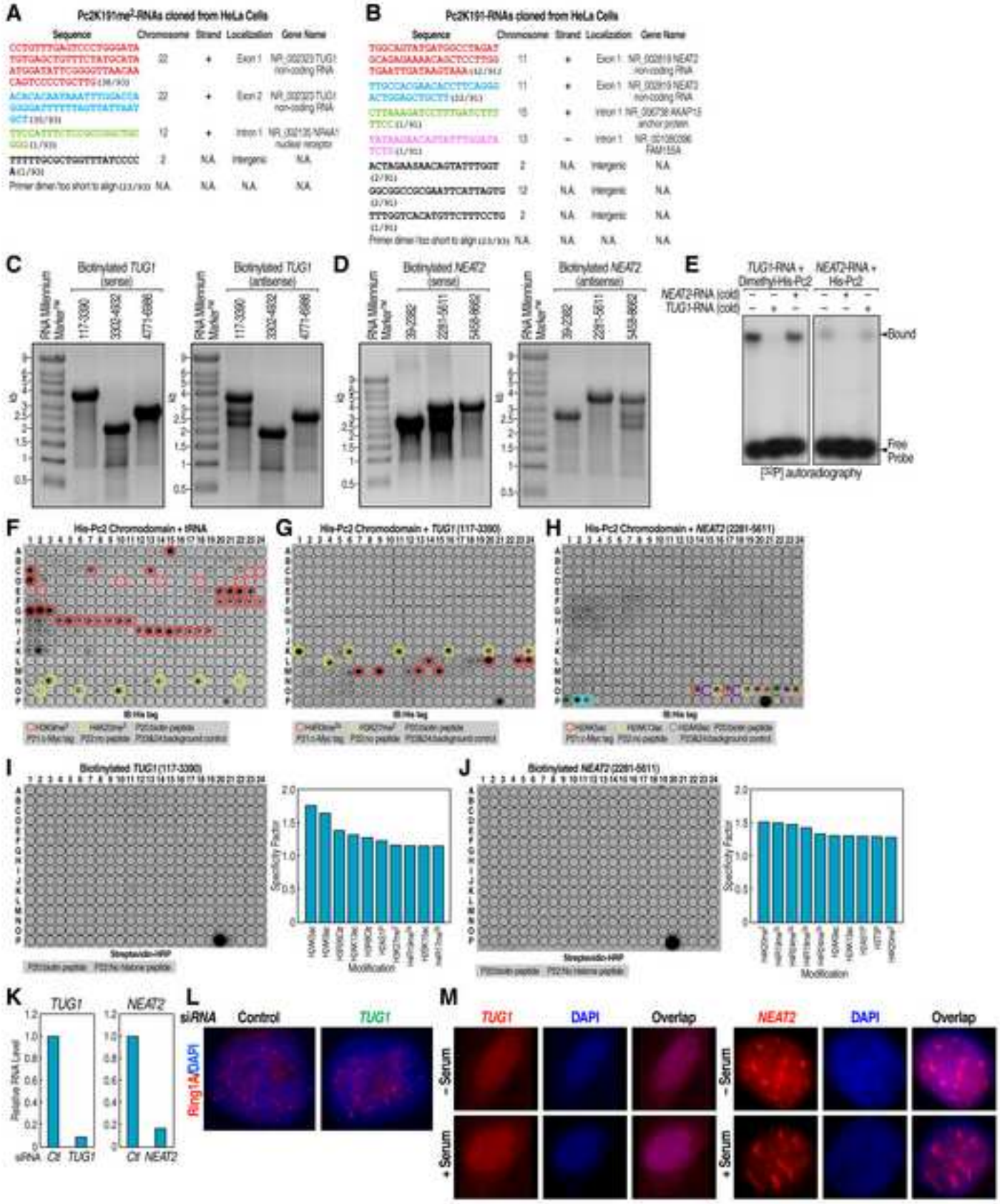

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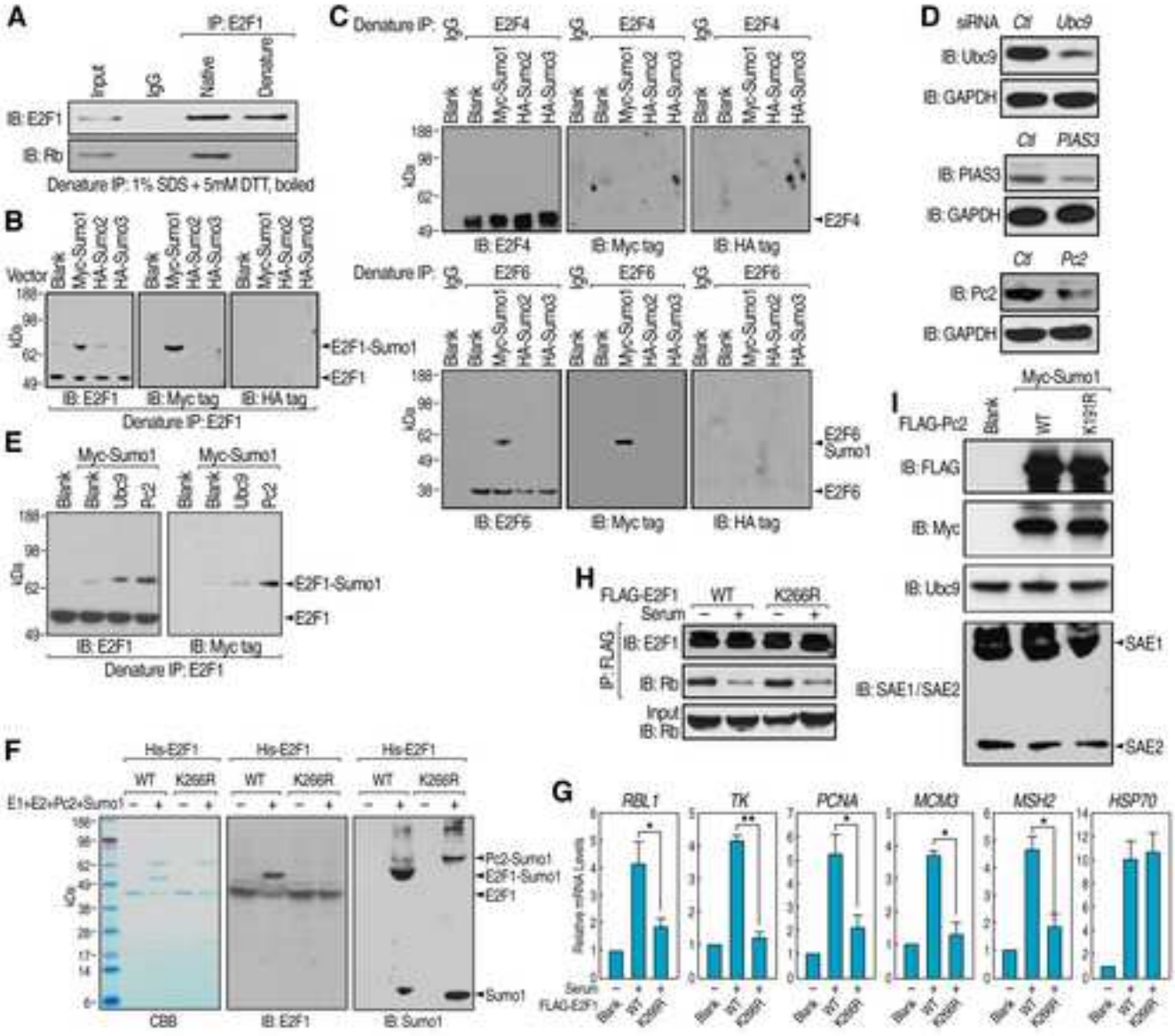

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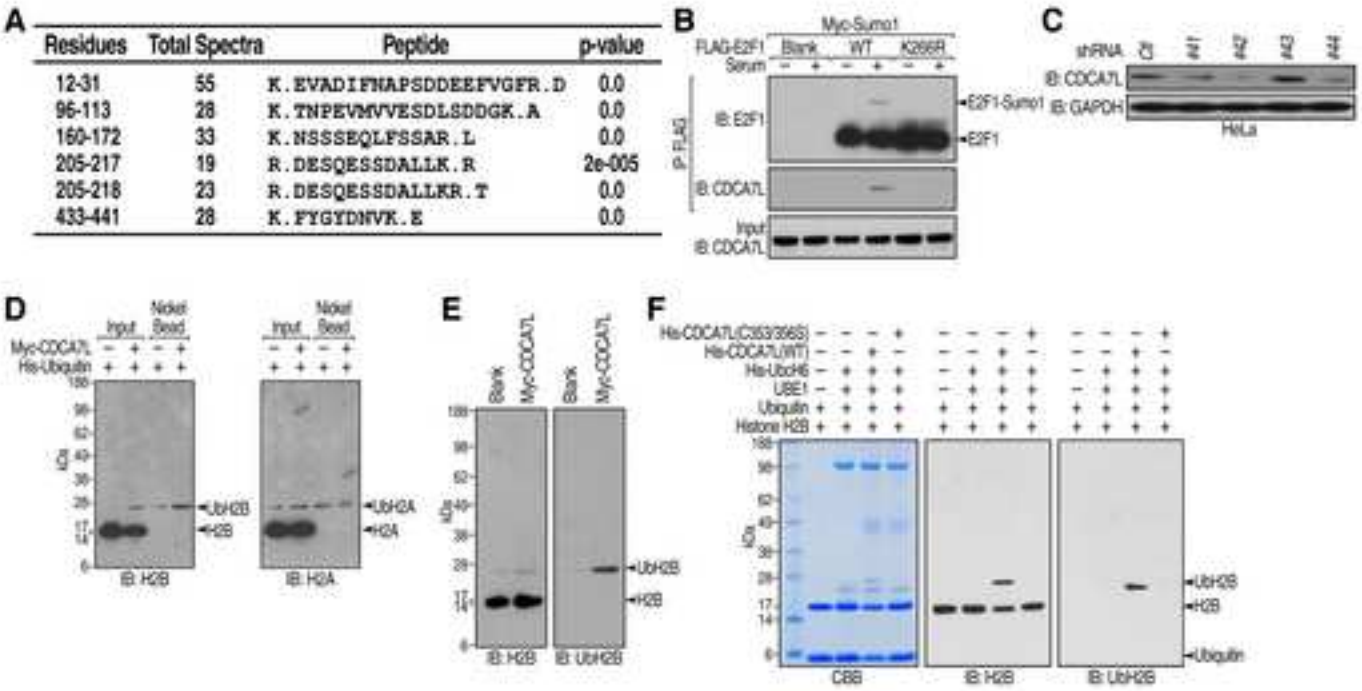
A

Residues	Total Spectra	Peptide	p-value
12-31	55	K.EVADIFNAPSDDEEFVGFR.D	0.0
96-113	28	K.TNPEVMVVESDLSDDGK.A	0.0
160-172	33	K.NSSSEQLFSSAR.L	0.0
205-217	19	R.DESQESSDALLK.R	2e-005
205-218	23	R.DESQESSDALLKR.T	0.0
433-441	28	K.FYGYDNVK.E	0.0



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A Chartered Journey for Growth Control Gene Activation

Eukaryotic nuclei contain distinct subsets of structures that associate with noncoding RNAs. In this issue, Yang et al now reveal that the noncoding RNAs *TUG1* and MALAT1/*NEAT2* control the signal-induced relocation of growth control genes between Polycomb bodies and interchromatin granules in the three dimensional space of the nucleus by selectively interacting with the promoter-associated methylated and unmethylated forms of Pc2, respectively. This provides a fresh insight into the link between subnuclear structure-specific noncoding RNAs and transcriptional regulation.