Supplementary Material and Methods

Liquid chromatography mass spectrometry

Proteins eluted from the DNA affinity columns were separated on 4-12 % Bis-Tris SDS gels (NuPAGE, Invitrogen) and stained either with silver or colloidal Coomassie. The gels were sliced into six to 8 equally sized gel pieces and subjected to tryptic in-gel digestion, essentially as described (Shevchenko et al. 2006). Prior to LC-MS analysis tryptic peptide mixtures were desalted using STAGE tips as described previously (Rappsilber et al. 2003; Rappsilber et al. 2007) with the following modifications. In order to ensure recovery of highly hydrophilic peptides, flow-through fractions from the C18 STAGE tips were further applied to Carbon STAGE tips (made from Empore 3M, activated carbon discs) and the pooled elutions from the C18 and Carbon tips were subsequently analyzed by LC-MS. For the AP2 column experiments nanoscale-LC (Agilent 1100 nanoflow system) was coupled to a QSTAR-Pulsar (ABI-Sciex) quadrupole time-of-flight hybrid mass spectrometer. Peptides were eluted from an analytical column by a linear gradient running from 5 to 60% (v/v) acetonitrile (in 0.5% acetic acid) with a flow rate of 200 nl/min in 90 minutes and sprayed directly into the orifice of the mass spectrometer. The 15 cm fused silica emitter with an inner diameter of 75 µm (New Objective, USA and Proxeon Biosystems, Denmark) was packed in-house with reverse-phase ReproSil-Pur C18-AQ 3 µm resin (Dr. Maisch GmbH, Germany). Proteins were identified by information-dependent acquisition of fragmentation spectra of doubly, triply or quadruply charged peptides with a precursor selection window of m/z = 350 to 1400 using pulsed extraction of fragments enhancing at m/z = 600. Tandem mass spectra were acquired for 1.5 s and fragmented peptides were excluded from sequencing for 90 s (Schulze and Mann 2004).

The other experiments were analyzed by nanoscale-LC (Agilent 1100 nanoflow system) coupled to a 7-Tesla linear ion-trap Fourier-transform ion cyclotron resonance mass spectrometer (LTQ-FT, Thermo Electron, Germany) equipped with a nanoelectrospray source (Proxeon, Denmark). Peptides were eluted from an analytical column by a linear gradient running from 2 to 50% (v/v) acetonitrile (in 0.5% acetic acid) with a flow rate of 250 nl/min in 90 minutes and sprayed directly into the orifice of the mass spectrometer. Information dependent acquisition of MS, MS/MS and MS^3 spectra was performed essentially as described with minor modifications (Olsen and Mann 2004). Survey full-scan MS spectra (m/z 350 – 1600) were acquired in the Fourier transform ion cyclotron resonance (FT-ICR) cell with resolution R = 25,000 at m/z = 400 with a target value of 5,000,000 ions allowing a maximum fill-time of 2000 ms. The three most intense ions were sequentially isolated for SIM (selected ion monitoring) scans with a 12 Th mass window, R = 50,000 and a target accumulation value of 50,000 (maximum fill-time = 500 ms). The same ions were fragmented in the linear ion trap by CID (collision induced dissociation) at a target value of 10,000 (maximum fill-time = 250 ms). For MS^3 the most intense ion (>350 Th) from each MS^2 spectrum was further isolated and fragmented. Target ions selected for MS^2 were dynamically excluded for 90 s. Total cycle time was 3 to 3.5 s. The general mass spectrometric conditions were: spray voltage, 2.3 kV; no sheath and auxiliary gas flow; ion transfer tube temperature, 120°C; collision gas pressure, 1.3 mTorr; normalized collision energy using wide-band activation mode; 30% for MS^2 and 28% for MS^3. Ion selection thresholds were 500 counts for MS^2 and 5 counts for MS^3. An activation q = 0.25 and activation time of 30 ms was applied in both MS^2 and MS^3 acquisitions.

Data analysis

MS data acquired on the QSTAR-Pulsar instrument were searched with Mascot 2.0 (Matrix Science, UK) against the human International Protein Index protein database (IPI, version 3.04, http://www.ebi.ac.uk/IPI/IPIhelp.html), to which we added frequently observed contaminants as described (Ong et al. 2004). The maximum allowed mass deviation MMD (Zubarev and Mann 2007) for monoisotopic precursor ions and MS/MS peaks was restricted to 0.1 Da in each case. Enzyme specificity was set to trypsin (with a maximum of 2 missed cleavages), allowing for cleavage N-terminal to proline and between aspartic acid and proline (Olsen et al. 2004). Carbamidomethyl cysteine was set as fixed and oxidized methionine, N-acetylation and $^{2}H_{4}$-lysine as variable modification. Protein identifications were further analyzed and manually verified by the use of MSQuant (http://msquant.sourceforge.net/).

FT-MS data were analyzed by MaxQuant version 1.0.6.15 (Cox and Mann submitted) and processed with lysine-d4 ($^{2}H_{4}$-lysine) labeling and standard settings for LTQFT. Enzyme specificity was set to trypsin, allowing for cleavage N-terminal to proline and between aspartic acid and proline. Carbamidomethyl cysteine was set as fixed and oxidized methionine, N-acetylation, as well as loss of ammonia from N-terminal glutamine as variable modifications. The pre-analyzed MaxQuant data was searched against Human International Protein Index protein sequence database (IPI, version 3.24) supplemented with frequent contaminants and concatenated with reversed copies of all sequences using Mascot (version 2.1.04, Matrix Science) with an MMD (Zubarev and Mann 2007) of 30 ppm for monoisotopic precursor ions and 0.5 Da for MS/MS peaks. Identified peptides were further analyzed and re-quantified with MaxQuant software. The required false positive rate was set to 0.05 at the peptide level and the required false discovery rate was set to 0.01 at the protein level with the minimum required peptide length set to 6 amino acids.

In addition to the protein false discovery rate threshold, proteins were only considered to be identified with at least two different (at sequence level) peptides, thereof one can be uniquely assigned to the respective protein sequence.

For the interpretation of MS^3 spectra, peak lists derived from Xcalibur 1.4 (Thermo Fisher Scientific) raw files were generated by software developed in-house (DTA Super Charge; http://msquant.sourceforge.net/), searched with Mascot (version 2.0) against the human IPI database (version 3.04) and analyzed by the MS^3 scoring function of MSQuant as described (Olsen and Mann 2004).

Normalized protein quantification values were only calculated for proteins that could be quantified with at least two MaxQuant-compatible SILAC-pairs associated with them. The extracted peptide ion current dependent (a measure for the protein abundance) statistical significance of protein ratios was further calculated with MaxQuant (significance “B”) as described (Cox and Mann submitted; Graumann et al. 2007). This is because for highly abundant proteins the statistical spread of unregulated proteins is much more focused than for low abundant ones, which is due to the fact that intense signals (high signal-to-noise ratio in mass spectrum) can be measured more accurate than less intense ones (low signal-to-noise ratio). Therefore, a protein that shows, for instance, a ratio of two should be very significant when it is highly abundant, while at very low abundance it should only be marginally significant. We defined ratios to represent highly significant “outliers” from the main distribution of all ratios (in one experiment) if they exhibited a distance of more than three standard deviations from the center of the distribution (significance < 0.0012) and considered these proteins to constitute specific interaction partners. Finally, we assessed the accuracy of protein ratio by calculating the coefficient of variability over all redundant quantifiable peptides.

DNA affinity chromatography experiments

Single-stranded HPLC-purified oligonucleotides (DNA Technology, Aarhus, Denmark) bearing a spacer sequence (5´CAAAA3` at the sense strand) followed by a PstI restriction site (5´CTGCAG3´) are reconstituted at a concentration of 50 nmol/ml in annealing buffer (20 mM Tris-HCl, pH 8.0, 10 mM $MgCl_2$, 0.1 M KCl). Complementary strands were annealed with the minus strand in slight excess to the biotinylated plus strand to ensure the exclusive immobilization of double stranded DNA molecules to streptavidin beads. Therefore, 0.03 ml of the sense strand oligonucleotide harboring the 5´Biotin-TEG spacer arm are mixed with 0.04 ml of the complementary (non biotinylated) oligonucleotide, heated for 5 min at 90°C and gradually cooled to 65°C (10 min). After an incubation step at 65°C for 5 min, the thermoblock is switched-off and allowed to cool to room temperature (the double-stranded DNA can be stored for several months at -20°C at this step). The biotinylated dsDNA (~250 pmol) is then diluted in buffer DW (20 mM Tris-HCl, pH 8.0, 2 M NaCl, 0.5 mM EDTA, 0.03% NP-40) to give a final volume of 0.4 ml and incubated (rotary wheel, 3 hrs, room temperature) with 1 mg of Dynal MyOne (Invitrogen) streptavidin magnetic beads that have been equilibrated with two washes (0.4 ml each) of TE buffer containing 0.01 % (v/v) NP40 and two washes of buffer DW (0.75 ml each). After the binding step the beads are washed one time in 0.4 ml TE (containing 0.02 % NP40), three times in 0.4 ml DW buffer and resuspended in 0.1 ml of buffer DW. At this step the DNA affinity column (~200 pmol/mg) can be stored for several weeks in the refrigerator.

Before the DNA affinity pull-down experiment the beads harboring the immobilized dsDNA are incubated for one hour in blocking buffer (20 mM Hepes, pH 7.9, 0.05 mg/ml BSA, 0.05 mg/ml glycogen, 0.3 M KCl, 0.02 % NP40, 2.5 mM DTT, 5 mg/ml polyvinylpyrrolidone) at RT on a rotary wheel using 1.3 ml buffer per mg beads. Excess blocking buffer is removed by washing the beads with 1.3 ml (per mg beads)

1x restriction endonuclease buffer (New England Biolabs buffer 3) containing 0.02 % NP40 and two times with 2.67 ml buffer G (20 mM Tris-HCl, pH 7.3, 10 % (v/v) glycerol, 0.1 M KCl, 0.2 mM EDTA, 10 mM potassium glutamate, 0.04 % NP40, 2 mM DTT, 0.4 mM PMSF and 0.005 mg/ml each of aprotinin, leupeptin and pepstatin A) per mg beads. Nuclear extracts from $^{2}H_{4}$-lysine labeled and unlabeled cells that have been stored at -80°C are cleared for insoluble matter by centrifugation at 15,000xg for 20 min in a benchtop centrifuge at 4°C. Cleared labeled and unlabeled nuclear extracts (~6-10 mg/ml) are adjusted to 10 mM potassium glutamate and quickly diluted with on volume of buffer G containing either 0.2 mg/ml of poly dIdC (for GC-rich DNA elements) or poly dAdT (for AT-rich DNA elements and the methyl-CpG baits). Again, insoluble matter is removed by centrifugation at 15,000xg for 10 min in a benchtop centrifuge at 4°C and the diluted extracts are separately subjected to a pre-clearing step (one hour, rotating wheel, cold lab) employing MyOne streptavidin magnetic beads (washed with TE/0.02% NP40, buffer DW and equilibrated in buffer G) at a final concentration of 1.5 mg/ml. The beads are removed with a magnetic separator and the labeled and unlabeled extracts are mixed separately with the “blocked” probe and the control DNA magnetic beads at a final concentration of 0.7 mg/ml, followed by a 3 hr incubation step (cold lab, rotating wheel). Magnetic beads are recovered (separator) and subjected to four washes with buffer G (1.8 ml per mg beads) and one wash (0.9 ml per mg beads) with restriction endonuclease buffer (NEB buffer 3; adjusted to 0.02% (v/v) NP40, 2.5% (v/v) glycerol, 1 mM DTT, 0.2 mM PMSF and 0.005 mg/ml each of aprotinin, leupeptin and pepstatin A). The probe and control beads are immediately combined using restriction endonuclease buffer and suspended at a concentration of 3.75 mg/ml followed by the addition of 0.014 ml of PstI (New England Biolabs) and a one hour incubation at 25°C in an Eppendorf thermomixer (at 1400 rpm).

This step is repeated once, the combined elutions are concentrated by ultrafiltration (Millipore Y3K, 8,000xg, 10°C, benchtop centrifuge) and the concentrated sample is adjusted to 1x LDS sample buffer/15 mM beta-mercaptoethanol (Invitrogen) directly in the ultrafiltration device.

Supplementary Figure and Table Legends

Figure S1. Restriction endonuclease cleavage is an efficient method to release the immobilized DNA. A slight excess of biotinylated double stranded DNA oligonucleotides is bound to streptavidin beads. Equal volumes of input and flow-through (FT) were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. Twenty percent of eluted material (Elution) from the experiment shown in Figure 2 (main text) was analyzed by agarose gel electrophoresis after isolation of DNA by phenol-chloroform extraction.

Figure S2. Identified tryptic peptides demonstrating the binding of TFAP2A (AP2-alpha) and TFAP2C (gamma) to the AP2 site containing DNA (see Figure 1 in main text). Observed tryptic peptides matching to AP2 family members are superimposed on a multiple alignment (ClustalW, http://www.ebi.ac.uk/Tools/clustalw/index.html) of TFAP2A (AP2A_HUMAN), TFAP2B (AP2B_HUMAN), TFAP2C (AP2C_HUMAN), TFAP2D (AP2D_HUMAN) and TFAP2E (AP2E_HUMAN). Shared peptides are highlighted in red, whereas TFAP2A- and TFAP2C-specific ones are illustrated in blue and green, respectively. Lysine and arginine residues amino-terminal to the trypsin cleavage site are highlighted in bold italics.

Figure S3. Representative peptide mass spectra demonstrating the preferential binding of UHRF1 (ICBP90), USP7, DNMT1, KIAA0101, ACTL8 (actin-like protein 8) and PCNA to the DNA column bearing the methylated *MTA2* CpG island. Representative MS spectra of labeled (monoisotopic peak marked with filled circle) and unlabeled (monoisotopic peak marked with open circle) tryptic peptides are shown.

The unlabeled peptide isotope cluster represents the relative binding of the proteins to the non-methylated DNA sequence, whereas the labeled peptide isotope cluster represents the relative binding to the methylated CpG island. A peptide mass spectrum derived from a tryptic peptide identifiying the RNA polymerase II POLR2E (p23) subunit is shown as a control for unspecific interactions with the DNA column.

Figure S4. Hypothetical model explaining the methylation-specific interaction of UHRF1/ICBP90, DNMT1, PCNA, KIAA0101, USP7 and ZBTB33/Kaiso with the *MTA*2 CpG island. UHRF1 and ZBTB33 bind directly (indicated by arrowheads) to the methylated (indicated by filled circles) CpGs, whereas the other proteins are most likely recruited by protein-protein interactions (see Figure 4 and Table 2 in main text). Briefly, DNMT1 directly interacts with PCNA (Chuang et al. 1997), UHRF1 (Achour et al. 2007; Bostick et al. 2007; Sharif et al. 2007) and KIAA0101 (Simpson et al. 2006; Yu et al. 2001). UHRF1 possesses ubiquitin E3 ligase activity (modifies histone H3) *in vitro* (Citterio et al. 2004) and contains six copies of a signature motif (consensus P/AXXS) that mediates the contact between USP7 and its target molecules (Sheng et al. 2006). USP7, an ubiquitin protease binds to and stabilizes E3 ligases by preventing their auto-ubiquitination (Canning et al. 2004). Since UHRF1 was recently shown to also read out the H3 K9 di- and tri-methylation mark by virtue of its PHD finger domain (Karagianni et al. 2007) our pull-down data corroborate the idea that UHRF1 is a central player coordinating maintenance and interpretation of DNA and histone methylation marks

Table S1. Proteins interacting specifically with the DNA column bearing a functional AP2 site. The Uniprot accession number, the molecular weight (MW), the ratio, the standard deviation (STDEV) and the number of quantified peptides (Q. Peptides) for specific binders are shown.

Table S2. Proteins exhibiting preferential binding to the *ESRRA* promoter hormone response element. The Uniprot accession number, the normalized ratio, the ratio variability, the quantification significance (ratio significance) and the number of unique peptides are shown.

Supplementary References

Achour, M., Jacq, X., Ronde, P., Alhosin, M., Charlot, C., Chataigneau, T., Jeanblanc, M., Macaluso, M., Giordano, A., Hughes, A.D. et al. 2007. The interaction of the SRA domain of ICBP90 with a novel domain of DNMT1 is involved in the regulation of VEGF gene expression. *Oncogene* **27:** 2187-2197.

Bostick, M., Kim, J.K., Esteve, P.O., Clark, A., Pradhan, S., and Jacobsen, S.E. 2007. UHRF1 plays a role in maintaining DNA methylation in mammalian cells. *Science* **317:** 1760-1764.

Canning, M., Boutell, C., Parkinson, J., and Everett, R.D. 2004. A RING finger ubiquitin ligase is protected from autocatalyzed ubiquitination and degradation by binding to ubiquitin-specific protease USP7. *J. Biol. Chem.* **279:** 38160-38168.

Chuang, L.S., Ian, H.I., Koh, T.W., Ng, H.H., Xu, G., and Li, B.F. 1997. Human DNA-(cytosine-5) methyltransferase-PCNA complex as a target for p21WAF1. *Science* **277:** 1996-2000.

Citterio, E., Papait, R., Nicassio, F., Vecchi, M., Gomiero, P., Mantovani, R., Di Fiore, P.P., and Bonapace, I.M. 2004. Np95 is a histone-binding protein endowed with ubiquitin ligase activity. *Mol. Cell. Biol.* **24:** 2526-2535.

Cox, J. and Mann, M. submitted. High peptide identification rates and proteome-wide quantitation via novel computational strategies.

Graumann, J., Hubner, N.C., Kim, J.B., Ko, K., Moser, M., Kumar, C., Cox, J., Schoeler, H., and Mann, M. 2007. SILAC-labeling and proteome quantitation of mouse embryonic stem cells to a depth of 5111 proteins. *Mol. Cell. Proteomics* **7:** 672-683.

Karagianni, P., Amazit, L., Qin, J., and Wong, J. 2007. ICBP90, a novel methyl K9 H3 binding protein linking protein ubiquitination with heterochromatin formation. *Mol. Cell. Biol.* **28:** 705-717.

Olsen, J.V. and Mann, M. 2004. Improved peptide identification in proteomics by two consecutive stages of mass spectrometric fragmentation. *Proc. Natl. Acad. Sci. U.S.A.* **101:** 13417-13422.

Olsen, J.V., Ong, S.E., and Mann, M. 2004. Trypsin cleaves exclusively C-terminal to arginine and lysine residues. *Mol. Cell. Proteomics* **3:** 608-614.

Ong, S.E., Mittler, G., and Mann, M. 2004. Identifying and quantifying in vivo methylation sites by heavy methyl SILAC. *Nat. Methods* **1:** 119-126.

Rappsilber, J., Ishihama, Y., and Mann, M. 2003. Stop and go extraction tips for matrix-assisted laser desorption/ionization, nanoelectrospray, and LC/MS sample pretreatment in proteomics. *Anal. Chem.* **75:** 663-670.

Rappsilber, J., Mann, M., and Ishihama, Y. 2007. Protocol for micro-purification, enrichment, pre-fractionation and storage of peptides for proteomics using StageTips. *Nat. Protoc.* **2:** 1896-1906.

Schulze, W.X. and Mann, M. 2004. A novel proteomic screen for peptide-protein interactions. *J. Biol. Chem.* **279:** 10756-10764.

Sharif, J., Muto, M., Takebayashi, S., Suetake, I., Iwamatsu, A., Endo, T.A., Shinga, J., Mizutani-Koseki, Y., Toyoda, T., Okamura, K. et al. 2007. The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature* **450:** 908-912.

Sheng, Y., Saridakis, V., Sarkari, F., Duan, S., Wu, T., Arrowsmith, C.H., and Frappier, L. 2006. Molecular recognition of p53 and MDM2 by USP7/HAUSP. *Nat. Struct. Mol. Biol.* **13:** 285-291.

Shevchenko, A., Tomas, H., Havlis, J., Olsen, J.V., and Mann, M. 2006. In-gel digestion for mass spectrometric characterization of proteins and proteomes. *Nat. Protoc.* **1:** 2856-2860.

Simpson, F., Lammerts van Bueren, K., Butterfield, N., Bennetts, J.S., Bowles, J., Adolphe, C., Simms, L.A., Young, J., Walsh, M.D., Leggett, B. et al. 2006. The PCNA-associated factor KIAA0101/p15(PAF) binds the potential tumor suppressor product p33ING1b. *Exp. Cell Res.* **312:** 73-85.

Yu, P., Huang, B., Shen, M., Lau, C., Chan, E., Michel, J., Xiong, Y., Payan, D.G., and Luo, Y. 2001. p15(PAF), a novel PCNA associated factor with increased expression in tumor tissues. *Oncogene* **20:** 484-489.

Zubarev, R. and Mann, M. 2007. On the proper use of mass accuracy in proteomics. *Mol. Cell. Proteomics* **6:** 377-381.

Web site references

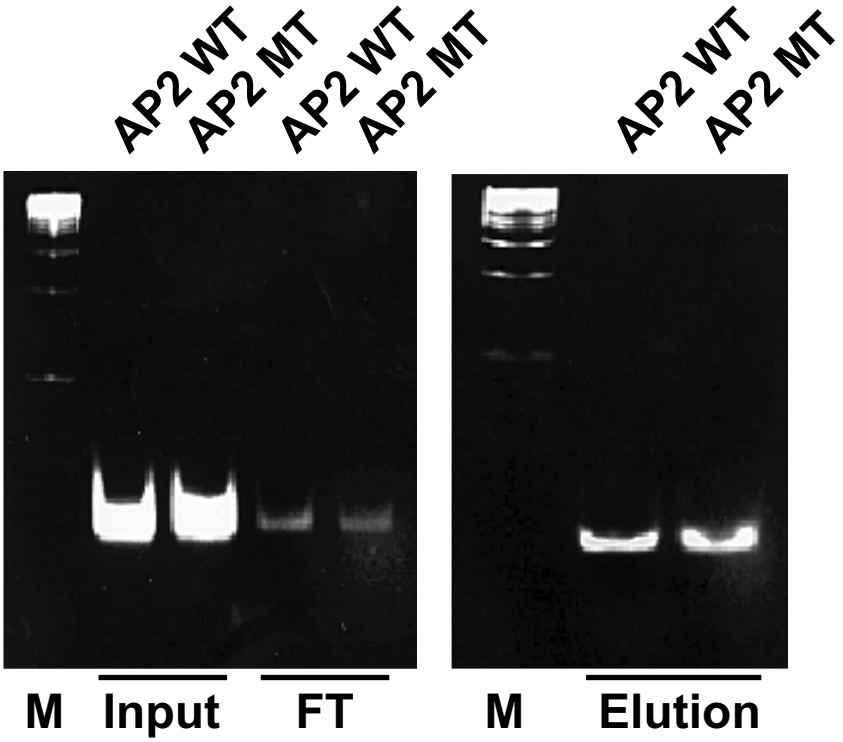
http://msquant.sourceforge.net/

http://www.ebi.ac.uk/IPI/IPIhelp.html

http://www.ebi.uniprot.org/index.shtml

http://www.ebi.ac.uk/Tools/clustalw/index.html

Figure S1



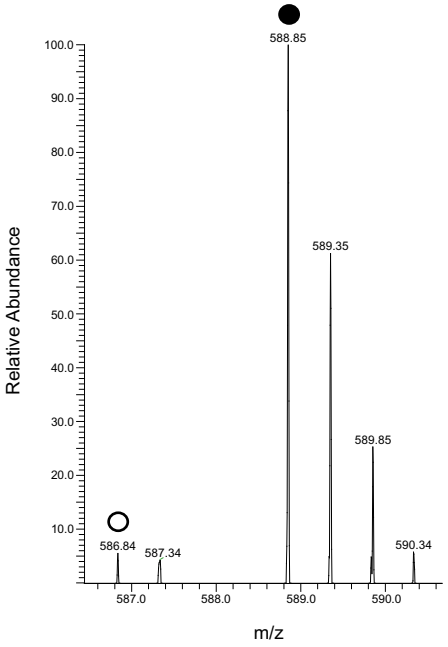
DNA agarose gel

Figure S2

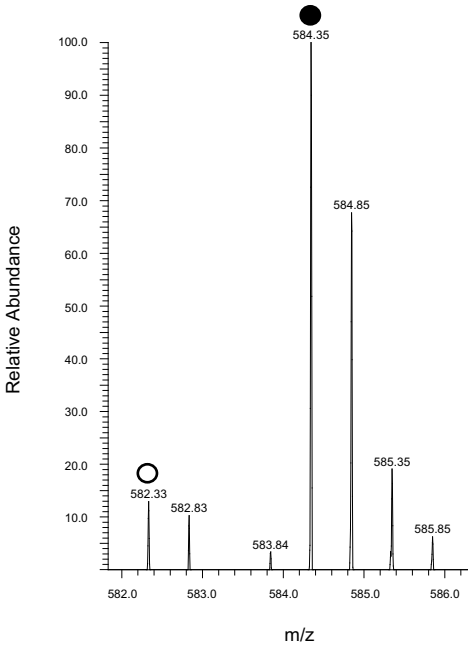

P05549|AP2A_HUMAN ----------MLWKLTDNIKYEDC-EDRHDGTSNGTARLPQLGTVGQSPYTSAPPLSHT 48
Q92481|AP2B_HUMAN MHSPPRDQAAIMLWKLVENVKYEDIYEDRHDGVPSHSSRLSQLGSVSQGPYSSAPPLSHT 60
Q92754|AP2C_HUMAN ----------MLWKITDNVKYEEDCEDRHDGSSNGNPRVPHLSSAGQHLYSPAPPLSHT 49
Q6VUC0|AP2D_HUMAN ----------MLVHTYSAME-------RPDGLG-AAAGGARLSSLPQAAYGPAPPLCHT 41
Q7Z6R9|AP2E_HUMAN ----------MSTTFPGLVHDAEIRHDGSNSYRLMQLGCLESVANSTVAYSSSSPLTYS 49
P05549|AP2A_HUMAN PNA----DFQPP-YFPPPY--QPI-YPQSQDP---YSHVN-DPYS--LNPLHAQPQP--Q 92
Q92481|AP2B_HUMAN PSS----DFQPP-YFPPPY--QPLPYHQSQDP---YSHVN-DPYS--LNPLHQ-PQ---Q 103
Q92754|AP2C_HUMAN GVA----EYQPPPYFPPPY--QQLAYSQSADP---YSHLG-EAYAAAINPLHQPAPTGSQ 99
Q6VUC0|AP2D_HUMAN PAATAAAEFQPP-YFPPPYPQPPLPYGQAPDAAAAFPHLAGDPYGG-LAPLAQPQPP--- 96
Q7Z6R9|AP2E_HUMAN TTG---TEFASP-YFSTNHQYTPL-HHQSFHYEFQHSHPAVTPDAYSLNSLHHSQQYYQQ 104
P05549|AP2A_HUMAN HPGWPGQRQ---SQESGLLHTHRGLPHQLSG-LDP-----RRDY---RRHEDLLHGP-HA 139
Q92481|AP2B_HUMAN HPWGQRQRQEVGSEAGSLLPQPRAALPQLSG-LDP-----RRDYHSVRRPDVLLHSAHHG 157
Q92754|AP2C_HUMAN QQAWPGRQSQEGAGLPSHHGRPAGLLPHLSG-LEAGAVSARRDAY--RRSDLLLPHAHAL 156
Q6VUC0|AP2D_HUMAN QAAWAAPRAAARAHEE--PPGLLAPPARALG-LDP-----RRDYA--TAVPRLLHGLADG 146
Q7Z6R9|AP2E_HUMAN IHHGEPTDFINLHNARALKSSCLDEQRRELGCLDAYR---RHDLS--LMSHGSQYGMHPD 159
P05549|AP2A_HUMAN LSSGLGD-LSIHSLPH--AIEEVPHVEDP---GINIPDQT-VIKKGPVSLSKSNSNAVSA 192
Q92481|AP2B_HUMAN LDAGMGDSLSLHGLGHP-GMEDVQSVEDANNSGMNLLDQS-VIKKVPVPP-----KSVTS 210
Q92754|AP2C_HUMAN DAAGLAENLGLHDMPH--QMDEVQNVDDQ---HLLLHDQT-VIRKGPISMT----KNPLN 206
Q6VUC0|AP2D_HUMAN AHGLADAPLGLPGLAAAPGLEDLQAMDEP---GMSLLDQS-VIKKVPIPSK---ASSLSA 199
Q7Z6R9|AP2E_HUMAN QRLLPGPSLGLAAAGA----DDLQGSVEAQ-CGLVLNGQGGVIRRG-------------- 200
P05549|AP2A_HUMAN IPINKDNLFGGV-VNPNEVFCSVPGRLSLLSSTSKYKVTVAEVQRRLSPPECLNASLLGG 251
Q92481|AP2B_HUMAN LMMNKDGFLGGMSVNTGEVFCSVPGRLSLLSSTSKYKVTVGEVQRRLSPPECLNASLLGG 270
Q92754|AP2C_HUMAN LPCQKE--LVGAVMNPTEVFCSVPGRLSLLSSTSKYKVTVAEVQRRLSPPECLNASLLGG 264
Q6VUC0|AP2D_HUMAN LSLAKDS-LVGGITNPGEVFCSVPGRLSLLSSTSKYKVTVGEVQRRLSPPECLNASLLGG 258
Q7Z6R9|AP2E_HUMAN ---------GTCVVNPTDLFCSVPGRLSLLSSTSKYKVTIAEVKRRLSPPECLNASLLGG 251
P05549|AP2A_HUMAN VLRRAKSKNGGRSLREKLDKIGLNLPAGRRKAANVTLLTSLVEGEAVHLARDFGYVCETE 311
Q92481|AP2B_HUMAN VLRRAKSKNGGRSLRERLEKIGLNLPAGRRKAANVTLLTSLVEGEAVHLARDFGYICETE 330
Q92754|AP2C_HUMAN VLRRAKSKNGGRSLREKLDKIGLNLPAGRRKAAHVTLLTSLVEGEAVHLARDFAYVCEAE 324
Q6VUC0|AP2D_HUMAN VLRRAKSKNGGRCLRERLEKIGLNLPAGRRKAANVTLLTSLVEGEAVHLARDFGYVCETE 318
Q7Z6R9|AP2E_HUMAN ILRRAKSKNGGRCLREKLDRLGLNLPAGRRKAANVTLLTSLVEGEALHLARDFGYTCETE 311
P05549|AP2A_HUMAN FPAKAVAEFLNRQHSD-PNEQVTRKNMLLATKQICKEFTDLLAQDRSPLGNSRPNPILEP 370
Q92481|AP2B_HUMAN FPAKAVSEYLNRQHTD-PSDLHSRKNMLLATKQLCKEFTDLLAQDRTPIGNSRPSPILEP 389
Q92754|AP2C_HUMAN FPSKPVAEYLTRPHLGGRNEMAARKNMLLAAQQLCKEFTELLSQDRTPHGTSRLAPVLET 384
Q6VUC0|AP2D_HUMAN FPAKAAAEYLCRQHAD-PGELHSRKSMLLAAKQICKEFADLMAQDRSPLGNSRPALILEP 377
Q7Z6R9|AP2E_HUMAN FPAKAVGEHLARQHME-QKEQTARKKMILATKQICKEFQDLLSQDRSPLGSSRPTPILDL 370
P05549|AP2A_HUMAN GIQSCLTHFNLISHGFGSPAVCAAVTALQNYLTEALKAMDKMYLS-----NNP-NSHTDN 424
Q92481|AP2B_HUMAN GIQSCLTHFSLITHGFGAPAICAALTALQNYLTEALKGMDKMFLN-----NTTTNRHTSG 444
Q92754|AP2C_HUMAN NIQNCLSHFSLITHGFGSQAICAAVSALQNYIKEALIVIDKSYMN-----PGD-QSPADS 438
Q6VUC0|AP2D_HUMAN GVQSCLTHFSLITHGFGGPAICAALTAFQNYLLESLKGLDKMFLS-----SVG-SGHGET 431
Q7Z6R9|AP2E_HUMAN DIQRHLTHFSLITHGFGTPAICAALSTFQTVLSEMLNYLEKHTTHKNGGAADSGQGHANS 430
P05549|AP2A_HUMAN N----AKSSDKEEKHRK----- 437
Q92481|AP2B_HUMAN EGP-GSKTGDKEEKHRK----- 460
Q92754|AP2C_HUMAN N-----KTLEKMEKHRK----- 450
Q6VUC0|AP2D_HUMAN K------ASEKDAKHRK----- 442
Q7Z6R9|AP2E_HUMAN EKAPLRKTSEAAVKEGKTEKTD 452

Figure S3

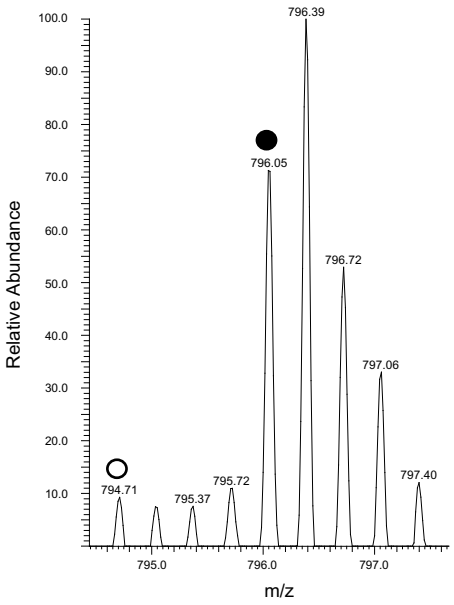
UHRF1 (ICBP90): LWNEVLASLK (2+)



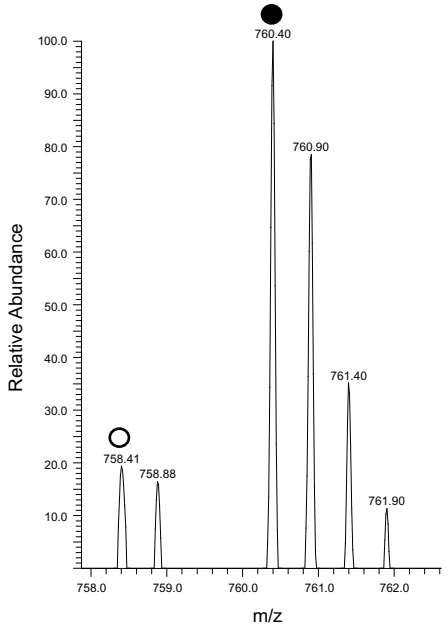
USP7: DLLQFFKPR (2+)



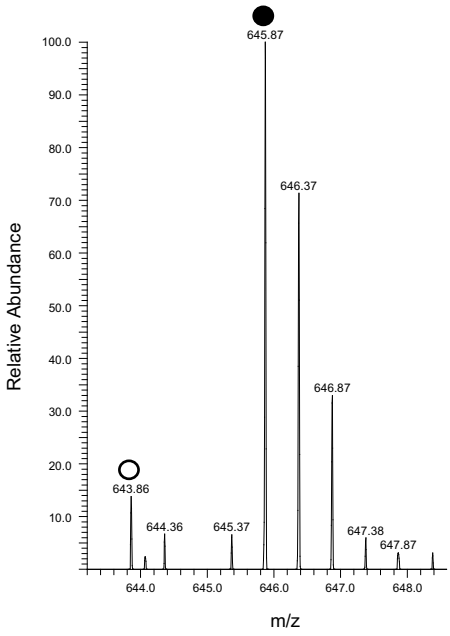
DNMT1: LSIFDANESGFESYEALPQHK (3+)



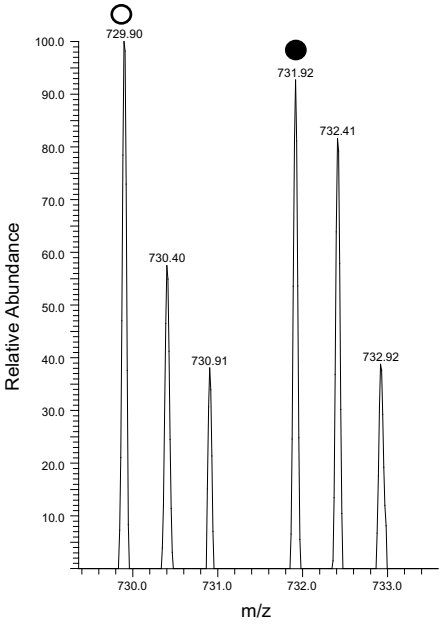
KIAA0101: YAGGNPKVCVRPTPK (2+)



ACTL8 (FLJ32777): TVIIDHGSGFLK (2+)



POLR2E: ALIVVQQGMTPSAK (2+)



PCNA: DLSHIGDAVVISCAK (2+)

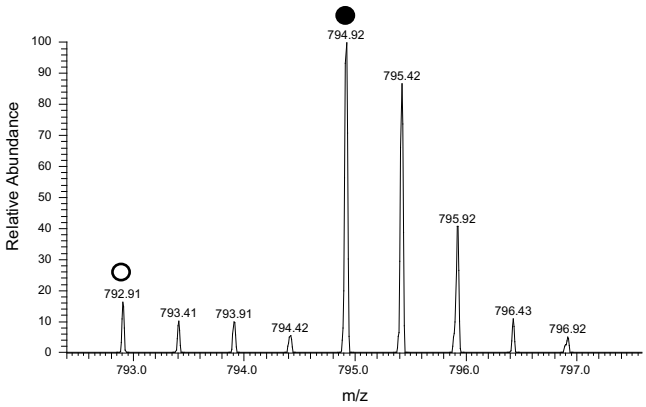


Figure S4

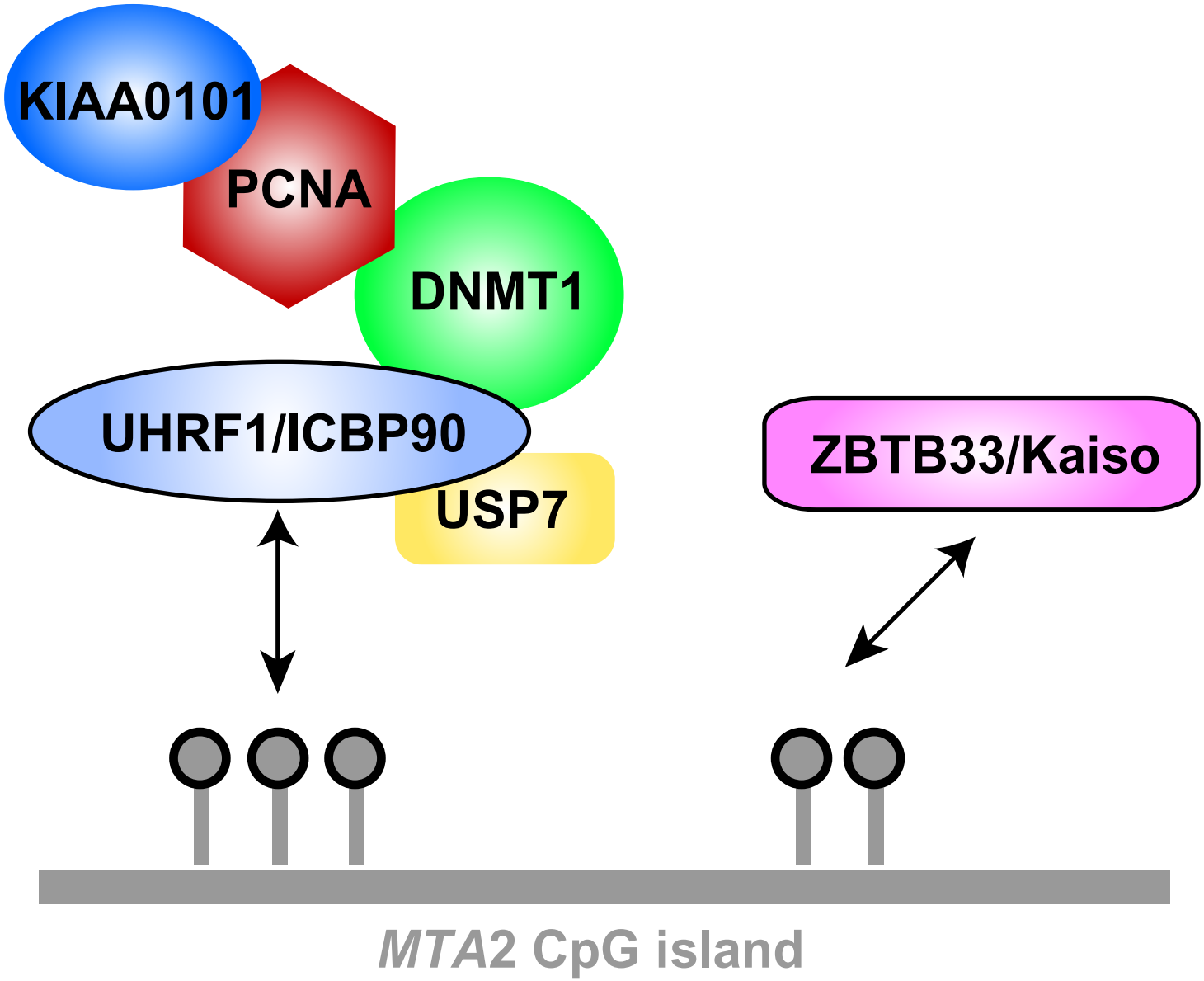

KIAA0101
PCNA
DNMT1
UHRF1/ICBP90
USP7
ZBTB33/Kaiso
MTA2 CpG island

Table S1

TFAP2 in-gel digestion

Protein Name	Uniprot	MW (kD)	Ratio	Quantified Peptides
TFAP2A	Q13777	48.06	6.75	10
TFAP2C	Q92754	49.18	5.00	4
PURA	Q00577	34.91	4.22	4
PURB	Q96QR8	33.24	6.73	11

Table S2

ESRRA in-gel digestion

Protein Name	Uniprot	Ratio Norm.	Ratio Significance	Unique Peptides	Quantified Peptides
ERR-alpha (ESRRA)	P11474	9.02	1.09E-43	18	14
NR2F6	P10588	4.93	3.56E-23	7	3
ATP citrate lyase (ACLY)	P53396	2.37	3.13E-09	3	2
Pur-beta (PURB)	Q96QR8	2.37	2.06E-07	3	2
Interleukin enhancer-binding factor 3 (ILF3)	Q12906-1	1.93	2.47E-06	5	5
Arsenite-resistance protein 2 (ARS2)	Q9BXP5-2	1.91	4.06E-06	2	2
TFII-I (GTF2I)	P78347-1	1.85	7.65E-06	12	6
Eukaryotic translation elongation factor 1 epsilon-1 (EEF1E1)	O43324	2.03	2.53E-05	5	4
KH-type splicing regulatory protein (KHSRP)	Q92945	1.68	1.44E-04	8	2
Ubiquitin-activating enzyme E1 (UBA1)	P22314	1.59	4.97E-04	9	3