

Table S1. Mass spectrometry analysis of proteins that copurified with MRGX, MRG15 long and short CHD from K562 nuclear extracts and after DNA damage (NCS) (Total spectral counts), Related to Fig. 1, S2A. Numbers in parenthesis are the spectral counts specific (not shared peptide sequence) for MRG15 or MRGX found in each other fraction.

NuA4/Tip60	MW	Mock	MRGX (Q15014)	MRG15		MRG15+NCS short CHD (Q9UBU8-2)
				long CHD (Q9UBU8-1)	Short CHD (Q9UBU8-2)	
TRRAP	436 kDa	0	174	184	392	547
EP400	336 kDa	0	175	143	301	348
BRD8	97 kDa	0	85	80	138	162
EPC1	91 kDa	0	22	16	36	53
EPC2	91 kDa	0	21	28	59	76
MBTD1	71 kDa	0	11	21	35	38
KAT5/TIP60	53 kDa	0	11	9	32	45
DMAP1	53 kDa	0	37	27	45	51
RUVBL2	51 kDa	0	61	58	93	106
RUVBL1	50 kDa	0	53	49	103	103
Baf53a	47 kDa	0	37	28	47	60
ING3	45 kDa	0	9	11	31	39
ACTB	42 kDa	0	30	30	38	47
Vps72	42 kDa	0	8	10	23	21
MRG15	40 kDa	0	17(2)	54	152	152
MRGX	32 kDa	0	138	6(1)	0	0
GAS41	27 kDa	0	20	17	21	22
MRGBP	22 kDa	0	21	9	34	42
MEAF6	22 kDa	0	5	7	10	8
H2B	14 kDa	0	2	2	8	8

Sin3B	MW	Mock	MRGX	long CHD	Short CHD	short CHD
KDM5A	196 kDa	0	3	9	29	36
EMSY	131 kDa	0	14	23	48	46
Sin3B	129 kDa	0	1	7	26	39
Pf1/PHF12	110 kDa	0	8	9	27	41
HDAC1	52 kDa	0	0	0	3	7
RBBP7	47 kDa	0	1	2	0	0

Others	MW	Mock	MRGX	long CHD	Short CHD	short CHD
BRCA2	384 kDa	0	8	16	60	83
ASH1L	332 kDa	0	0	2	27	33
PALB2	131 kDa	0	5	3	22	22
EP400NL	45 kDa	0	32	21	67	73
MRFAP1	15 kDa	0	12	3	6	2

Table S2. Mass spectrometry analysis of endogenous ASH1L purified from K562 cells (total spectral counts). Related to Figure 2.

Identified Proteins	MW	ASH1L (Q9NR48)
ASH1L	332 kDa	222
AKAP8	76 kDa	13
AKAP8L	72 kDa	7
RBBP4/ NuRF55	48 kDa	12
RBBP7	46 kDa	11
MRG15	40 kDa	17
MRGX	32 kDa	10

Table S3. Mass spectrometry analysis of MRG15 WT versus mutant interactomes purified from K562 cells through MRG15-3xFlag 2xStrep integrated at AAVS1 (total spectral counts). Related to Figures 3, S2B.

	Identified proteins	MW	MRG15				
			WT #1	WT #2	Y235A	W172A Y235A #1	W172A Y235A #2
NuA4/TIP60 complex	TRRAP	436 kDa	143	703	43	22	495
	EP400	343 kDa	240	462	210	61	349
	BRD8	103 kDa	105	231	86	35	173
	EPC2	91 kDa	51	137	34	14	82
	EPC1	91 kDa	29	71	23	7	49
	MBTD1	71 kDa	15	57	6	1	29
	KAT5	62 kDa	28	99	13	5	75
	DMAP1	53 kDa	52	92	17	14	69
	RUVBL2	51 kDa	166	203	79	34	108
	RUVBL1	50 kDa	90	199	62	18	151
	ACTL6A	47 kDa	45	66	30	16	48
	ING3	45 kDa	19	57	3	2	33
	ACTB	42 kDa	45	58	25	64	50
	VPS72	41 kDa	26	36	6	7	28
	MRG15	37 kDa	203	173	163	89	178
	YEATS4	27 kDa	15	33	4	4	20
	MRGBP	22 kDa	27	55	27	9	28
MEAF6	22 kDa	6	22	0	0	15	
TINTIN	EP400NL	52 kDa	62	72	39	14	59
Sin3B complex	EMSY	141 kDa	48	50	0	0	0
	PHF12/Pf1	110 kDa	22	42	0	0	0
	SIN3B	133 kDa	9	39	0	0	0
	KDM5A	192 kDa	9	54	0	0	0
	RBBP4	48 kDa	4	7	0	0	1
Other factors	BRCA2	384 kDa	21	71	0	0	0
	ASH1L	333 kDa	10	26	0	0	1
	PALB2	131 kDa	14	31	0	0	0
	MRFAP1	15 kDa	6	0	0	0	0

Table S4. Mass spectrometry analysis of NuA4/TIP60 complex and EP400NL that interact with MRGBP and BRD8 transcripts (with 2BRDs or all transcripts (N-term CRISPR) or 1BRD), purified from K562 cells (total spectral counts). Related to Figure 4.

Identified Proteins	MW	BRD8			MRGBP (Q9NV56)
		Endogenous	1 BRD (Q9H0E9-2)	2 BRD (Q9H0E9-1)	
TRRAP	436 kDa	330	110	33	408
EP400	340 kDa	275	139	42	340
BRD8	135 kDa	117	108	23	168
EPC2	91 kDa	68	31	5	63
EPC1	91 kDa	33	18	2	45
MBTD1	71 kDa	30	16	3	34
KAT5	53 kDa	20	13	0	29
DMAP1	53 kDa	59	32	7	60
RUVBL2	51 kDa	127	62	26	137
RUVBL1	50 kDa	84	35	18	118
ACTL6A	47 kDa	47	23	8	51
ING3	45 kDa	23	4	0	34
ATCB	42 kDa	46	28	11	49
VPS72	42 kDa	14	12	3	22
MRG15	40 kDa	44	54	7	251
MRGX	32 kDa	42	56	9	142
YEATS4	27 kDa	27	15	3	25
MRGBP	22 kDa	23	25	5	115
MEAF6	22 kDa	8	8	0	9
H2B	14 kDa	0	7	0	4
EP400NL	45 kDa	64	11	0	70

Table S5. Mass spectrometry analysis of FPLC gel filtration fractions 23 of BRD8 and MRGBP corresponding to the TINTIN complex and 33 of MRGBP corresponding to the MRGBP/MRG15 or MRGX dimer (total spectral counts). Related to Figure 4.

Identified Proteins	MW	BRD8	MRGBP	
		Fraction 23	Fraction 23	Fraction 33
BRD8	103 kDa	76	110	8
MRG15	41 kDa	9	39	15
EP400NL	38 kDa	23	56	3
MRGX	32 kDa	12	35	20
MRGBP	22 kDa	16	30	24

Table S6. Mass spectrometry analysis of EP400NL interactome purified from K562 cells through EP400NL-3xFlag-2xStrep integrated at AAVS1 or from CRISPR/Cas9-mediated tagging of the endogenous gene (total spectral counts). Related to Figure 5.

TINTIN	MW	EP400NL	
		CRISPR	Q6ZTU2-5 AAVS1
BRD8	103 kDa	192	313
EP400NL	52 kDa	95	133
MRG15	37 kDa	117	164
MRGX	32 kDa	71	107
MRGBP	22 kDa	41	63

mRNA processing factors	MW	EP400NL	
		CRISPR	Q6ZTU2-5 AAVS1
SRRM2	300 kDa	11	4
SF3B1	146 kDa	5	2
SF3B3	136 kDa	13	13
GEMIN4	119 kDa	2	0
U2SURP	118 kDa	6	6
SF3B2	100 kDa	7	8
RBM5	92 kDa	5	2
DDX20	92 kDa	2	1
HNRNPM	78 kDa	14	15
PRPF31	55 kDa	12	6
HNRNPK	51 kDa	7	4
HNRNPF	46 kDa	3	5
RBM17	45 kDa	2	4
HNRNPC	32 kDa	2	0
SNRPD3	14 kDa	2	1

Note: All protein identifications determined by mass spec of different fractions are included in supplementary tables within a **supplemental Excel file**, including accession numbers, spectral counts, unique peptides and percentage coverage.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

ChIP-sequencing Experiments

For Flag ChIP, 1mg of cross-linked chromatin from K562 cells was incubated with 10µg of anti-Flag antibody (Sigma, M2) pre-bound on 300 µl of Dynabeads Prot-G (Invitrogen) overnight at 4°C. The beads were washed extensively and eluted in 0.1% SDS, 0.1M NaHCO₃. Crosslink was reversed with 0.2M NaCl and incubation overnight at 65°C. Samples were treated with RNase and Proteinase K for 2h and recovered by phenol-chloroform and ethanol precipitation. Libraries for sequencing were prepared with TruSeq LT adaptors (Illumina). Samples were sequenced by 50 bp single reads on HiSeq 4000 platform (Illumina).

Processing, Alignment and Peak Calling of ChIP-seq Data

FastQ format reads were aligned to the hg19 human reference using the Bowtie alignment algorithm (1). Bowtie2 version 2.1.6 was used with the pre-set sensitive parameter to align ChIP sequencing reads. MACS version 2.0.10 (model-based analysis of ChIPseq) peak finding algorithm was used to identify regions of ChIP-Seq enrichment over the background (2). The pipeline, commands, and parameters that were used are: Trimming of sequence (filter out 39 adaptor, and remove last 2 bases and 3 extra bases if it matches with adaptor sequence). Mapping sequences to human genome (hg19) using Bowtie: (i) command: bowtie2 -p 8 -sensitive -x genome/genome -U sequence.reads.fastq -S sample.sam. Peak calling algorithm MACS: (i) command: macs2 callpeak -t ChIPseq.data.bam -c input.sample.file.bam --broad -f BAM -g hs -n [directory] --outdir MACS2_files --nomodel --shiftsize 100 -B. Unique mapped read values were normalized to library size. Peaks were annotated as per human genome EnsDb.Hsapiens.v75 - hg19. Raw sequences and processed data of ChIP-sequencing from K562 cells were deposited in the GEO database under accession number **GSE181533**.

Cell Cycle Analysis

Fluorescence-activated cell sorting (FACS) analysis was used for cell cycle profiling. The cells were harvested by trypsinization, fixed with 70% ethanol, treated with propidium iodide for FACS analysis on a BD Accuri C6 Plus.

Baculovirus expression

BRD8, MRG15 and MRGBP cDNA were cloned in pFastBac vectors, packaged in viral particles with DH10Bac competent cells, and used to co-infected Sf9 cells. 48hrs post-infection cells were harvested, protein extracted and incubated with anti-HA beads. After washes, HA peptides were used to elute HA-tagged MRGBP and its associated proteins.

1. Langmead, B., Trapnell, C., Pop, M., and Salzberg, S. L. (2009) Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol* **10**, R25
2. Feng, J., Liu, T., Qin, B., Zhang, Y., and Liu, X. S. (2012) Identifying ChIP-seq enrichment using MACS. *Nat Protoc* **7**, 1728-1740

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. MRG15 isoforms and MRGX binding to chromatin *in vitro* and *in vivo*, related to Figures 1 and 2.

(A) Amino acids sequence alignment of MRG15 isoforms that have a “long” or “short” chromodomain, the paralog MRGX without chromodomain, and the Eaf3 homolog from *Saccharomyces cerevisiae*. The chromodomain and hydrophobic cage necessary to bind methylated lysine 36 of histone H3 are indicated.

(B) Whole-cell extract of isogenic K562 cells stably expressing the indicated proteins with a 3xFlag-2xStrep tag from the *AAVS1* safe harbor locus or endogenously tagged (CRISPR MRGX).

(C) Anti-FLAG ChIP-seq analysis of MRG15-S/L and MRGX in K562 cells using the *AAVS1* isogenic cell lines. Venn diagram of mapped significant genes that are bound for each protein and overlaps. MRG15 short isoform (MRG15-S) and MRGX bind mostly the same loci. Only a very small number of bound loci was detected for the MRG15 long isoform. Numbers represent the number of significant genes in the vicinity of mapped peaks.

(D) Genomic editing with the CRISPR/Cas9 system for tagging the endogenous MRGX. Schematic of the *MRGX* locus, Cas9 target site, and donor construct used to insert the cassette containing 3xFlag-2xStrep in the last exon. Annotated are the positions of the stop codon target site, the PAM motif, and homology arms left and right (HAL and R).

(E) Schematic of the *ASH1L* locus, Cas9 target site, and donor construct used to insert the cassette containing 3xFlag-2xStrep in the last exon. Annotated are the positions of the stop codon target site, the PAM motif, and homology arms left and right (HAL and R).

Figure S2. MRG15 purification after DNA damage or carrying mutations and baculovirus expression of the BRD8-MRGBP-MRG15 trimer, related to Figures 1, 3, 4 and Tables S1, S3.

(A) Tandem affinity purified MRG15 (short) from K562 cells treated, or not, with 50ng/mL of neocarzinostatin (NCS) for 3h before being collected to prepare the nuclear extracts. Fractions were run on gel and silver stained.

(B) Tandem affinity purified wild-type (WT) or double mutant (W172A Y235A) MRG15 (short) from K562 cells. Fractions were run on gel and silver stained.

(C) Purification of reconstituted BRD8-MRGBP-MRG15 trimer from baculovirus. Sf9 cells were coinfecting with single viruses for 48h. Single HA immunopurification was followed by elution with HA peptides. Purified fraction was migrated on gel and stained with coomassie. (*contaminant).

Figure S3. Exponentially modified protein abundance index (emPAI) of MRG15, MRGBP, BRD8, MRGX and EP400NL purified fractions to evaluate ratio of interactors, related to Figures 1, 3, 4 and 5, Tables S1, S3, S4 and S6.

(A-C) emPAI of MRGBP vs MRG15, BRD8 vs MRG15 and MRGX vs MRG15. The higher abundance of MRGBP, BRD8 with MRG15/X supports the existence of a trimeric complex. RUVBL1/2 are also abundant since they form a hexameric ring within NuA4/TIP60.

(D) emPAI of MRGBP vs EP400NL to support the concept of a stoichiometric assembly of EP400NL-BRD8-MRGBP-MRG15/X.

Figure S4. CRISPR/Cas9-mediated tagging of endogenous BRD8 and EP400NL, related to Figures 4-5.

(A) Whole-cell extracts (WCE) were analyzed by immunoblotting with anti-Flag. Anti-KAP1 was used as loading control.

(B) Genomic editing with the CRISPR/Cas9 system for tagging the double bromodomain (2BRD) isoform BRD8 (at the C-term) and all BRD8 isoforms (at the N-term). Schematic of the *BRD8* locus, Cas9 target site, and donor construct used to insert the cassette containing 3xFlag-2xStrep in the last exon for 2BRD or first exon for all isoforms. Annotated are the positions of the stop/start codon target sites, the PAM motif, and homology arms left and right (HAL and R).

(C) Anti-Flag analysis of BRD8 whole-cell extracts from *AAVS1* and endogenously tagged cell lines. The N-terminal endogenous tagging reveals the main expression of the 1BRD isoform, and the C-terminal tagging fails to detect a clear signal. Even expression from *AAVS1* leads to low level of the 2BRD isoform.

(D) Protein sequence alignment to show the very high similarity/identity of the N-terminal region of human EP400 with the previously uncharacterized EP400NL protein.

(E) Genomic editing with the CRISPR/Cas9 system for tagging endogenous EP400NL in C-term. Schematic of the *EP400NL* locus, Cas9 target site, and donor construct used to insert the cassette containing 3xFlag-2xStrep in the last exon. Annotated are the positions of the stop codon target site, the PAM motif, and homology arms left and right (HAL and R).

(F) EP400NL expression from endogenously tagged or *AAVS1* K562 cells. Anti-GAPDH is used as loading control.

Figure S5. Depletion of TIP60 versus TINTIN subunits in U2OS cells, related to Figure 6.

(A) Validation by RT-qPCR of the different siRNAs used for depletion and RNA sequencing analyses. RPLP0 was used as control. Different sets of primers were annotated. Knockdowns (KDs) were normalized with the siControl (siLuciferase). Error bars represent the range of two independent experiments.

(B) Cell cycle profile analysis of U2OS cells 48hrs after transfection of the different siRNAs. Error bars represented the range of two independent experiments.

Statistical analyses were performed by two-way ANOVA test followed by Tukey's test, *, $p < 0.1$, **, $p < 0.01$, ***, $p < 0.0001$.

(C) 253 genes are downregulated (left) and 142 genes are upregulated (right) by KDs of TINTIN subunits (BRD8, MRGBP, and MRGX) but not NuA4/TIP60 complex. To highlight the genes with high changes in expression, a cutoff of 2-fold difference was applied to the $\text{Log}_2(\text{fold change})$ values between the selected KDs and control (siLuc).

(D) Gene ontology analysis for downregulated (left) and upregulated genes (right) showing a significant enrichment ($p\text{Value} < 0.01$) using DAVID 6.8.





