

Supplementary Figures and Tables

SETD6 lysine methylation of RelA couples GLP activity at chromatin to tonic repression of NF- κ B signaling

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Supplementary Table S1

	PKMT name	SET domain	Full length		PKMT name	SET domain	Full length
1	SETD2	+	+	23	SETDB1	+	+
2	SETD4	+	+	24	SETDB2	+	+
3	SETD5	+	+	25	GLP	+	+
4	SETD6	+	+	26	G9A	+	+
5	SET7/9		+	27	SETMAR	+	+
6	SETD8		+	28	ASH1	+	
7	PRDM1		+	29	SUV-39H1		+
8	PRDM2	+	+	30	SUV-39H2		+
9	PRDM3		+	31	SUV-420H1		+
10	PRDM4	+	+	32	SUV-420H2		+
11	PRDM5		+	33	ZNF298	+	
12	PRDM6		+	34	SMYD1		+
13	PRDM7		+	35	SMYD2		+
14	PRDM8	+	+	36	SMYD3		+
15	PRDM9		+	37	SMYD4		+
16	PRDM10		+	38	SMYD5		+
17	PRDM11		+	39	NSD1	+	
18	PRDM14	+	+	40	NSD2	+	+
19	PRDM15		+	41	NSD3	+	+
20	PRDM16		+	42	EZH1	+	+
21	PRDM17		+	43	EZH2	+	+
22	PRDM18		+				

Supplementary Table S1. Summary of human enzymes present in the PKMT library.

Supplementary Table S2

PKMT	aa	Expression sf9 cells	E.coli
SETD2 _{SET}	1483-1700		+
SETD4	1-440	+	
SETD5 _{SET}	1-394		+
SETD6	1-473	+	+
SETD7	1-366		+
SETD8	1-393		+
PRDM2 _{SET}	16-157		+
PRDM4 _{SET}	403-545		+
SETDB1	1-1291	+	
SETDB2	1-719	+	
GLP _{SET}	970-1267		+
G9a _{SET}	913-1210		+
SETMAR _{SET}	60-286		+
ASH _{SET}	2080-2299		+
SUV39H2	1-410		+

Supplementary Table S2. List of enzymes used in Figure 1a. SET- SET domain only; aa- amino acids.

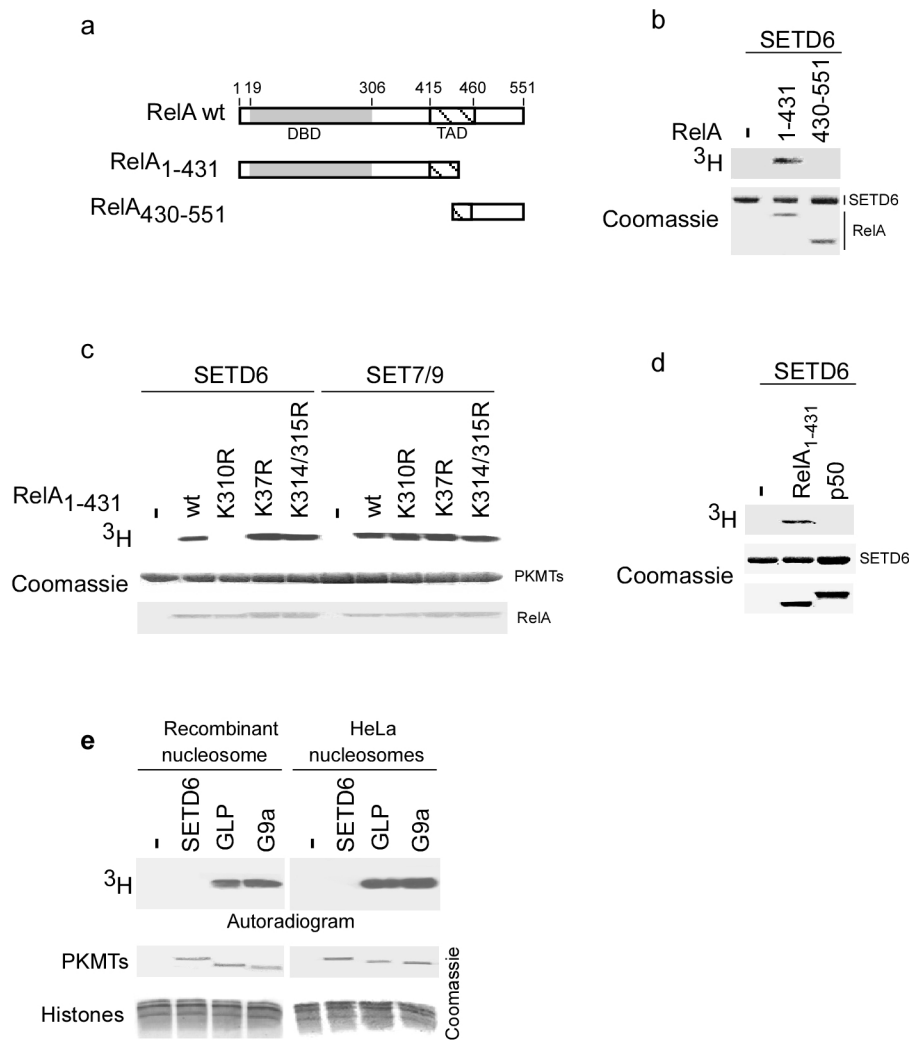
Supplementary Table S3

Primers used for ChIP analysis

	Target	Sequence	
Human	<i>IL8</i>	FW	TGATGACTCAGGTTTGCCCTG
		BC	ATCATCACCTACTAGAGAACTTATGC
	<i>IL1A</i>	FW	CCTCAAGTGATTGTCCTGCCTC
		BC	TTGGGTTCCCAGTTGGAGTT
	<i>MYC</i>	FW	ACGTCCGGTTTGTCGG
		BC	GGACTTCCTAAAAGGGGCAAG
	<i>CCND1</i>	FW	GAGGTGTGTTTCTCCCGGT
		BC	GGAGACTCTTCGGGCTGC
<i>GAPDH</i>	FW	CTACTAGCGGTTTTACGGGCG	
	BC	TCGAACAGGAGGAGCAGAGAGCGA	
Mouse	<i>Il6</i>	FW	AAGGTTTCCAATCAGCCCCAC
		BC	GCAGAATGAGCTACAGACATCCC
	<i>Tnf</i>	FW	CTTCTGAAAGCTGGGTGCATAAG
		BC	CCATGCACACTTCCCAACTCT
	<i>Ccnd1</i>	FW	CCGGCTTTGATCTCTGCTTA
		BC	GCTGTACTGCCGGTCTCC
	<i>Myc</i>	FW	GGA ACTTACAATCTGCGAGCCA
		BC	CTTCAAACAGCTCGAGGAGCTC

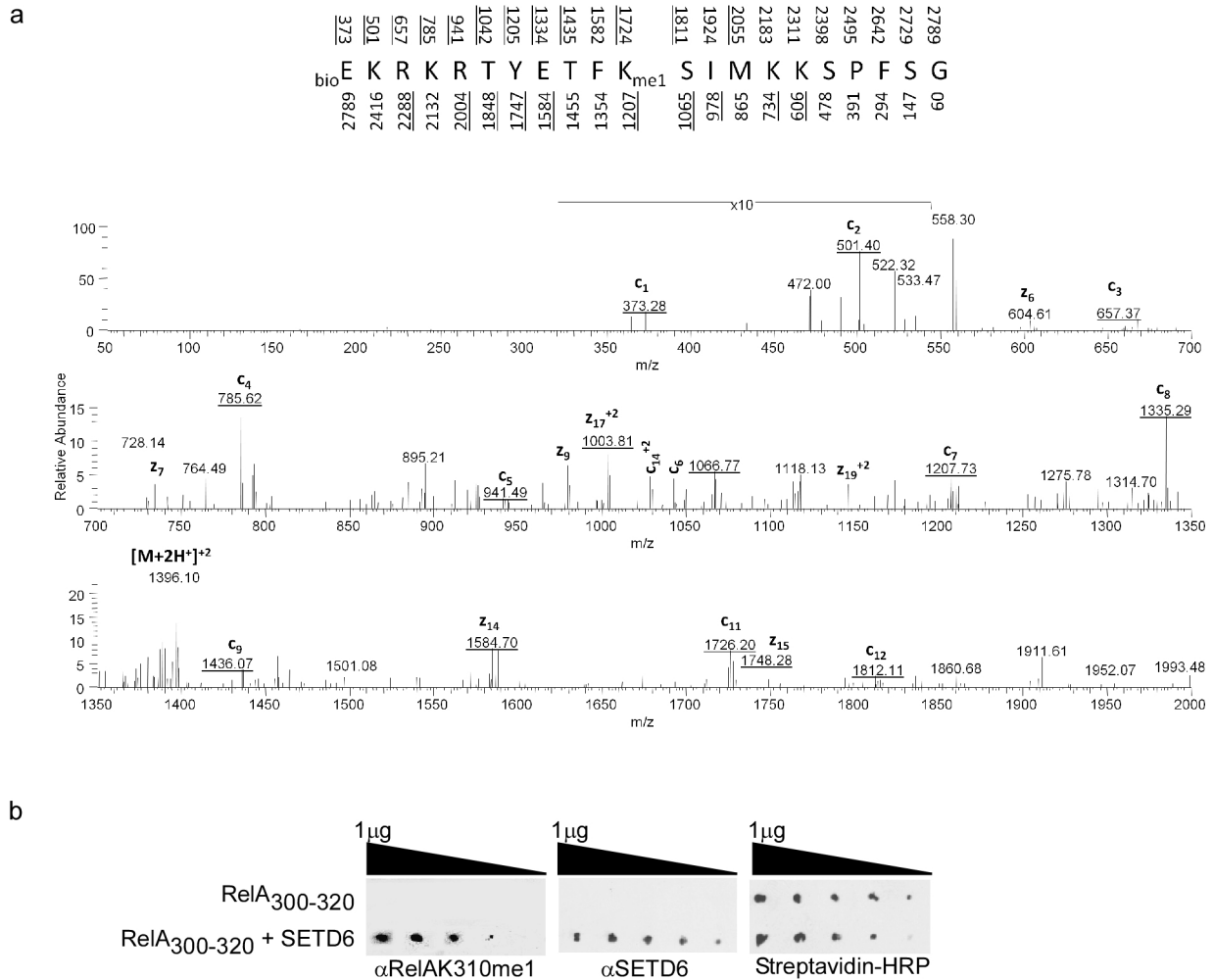
Supplementary Table S3. List of human and mouse primers used for the ChIP assays.

Supplementary Figure S1



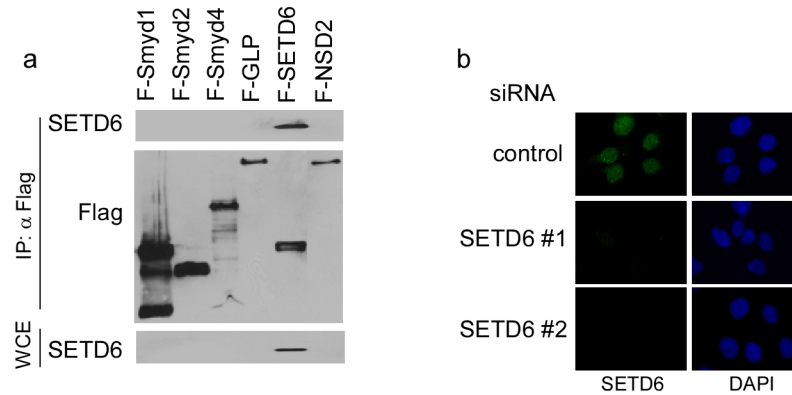
Supplementary Figure S1. SETD6 specifically methylates RelA at K310. a, Schematic of full length RelA, RelA₁₋₄₃₁ and RelA₄₃₀₋₅₅₁. DBD: DNA binding domain; TAD: Transactivation domain. **b,** SETD6 methylates an N-terminal RelA (RelA₁₋₄₃₁) but not a C-terminal (RelA₄₃₀₋₅₅₁) polypeptide. Autoradiogram of methylation reactions with recombinant RelA constructs and recombinant SETD6. Coomassie stain of recombinant proteins used in the reactions is shown below the autoradiogram. Data represent a single experiment **c,** SET7/9 does not methylate RelA at K310. Autoradiogram of SETD6 and SET7/9 methylation reactions on recombinant wild-type (wt) RelA₁₋₄₃₁ and the indicated RelA mutants. Coomassie stain of recombinant proteins used in the reactions is shown below the autoradiogram. Data represent duplicate experiments. **d,** SETD6 does not methylate p50. Autoradiogram of SETD6 methylation reactions on recombinant RelA₁₋₄₃₁ and p50. Coomassie stain of recombinant proteins used in the reactions is shown below the autoradiogram. Data represent a single experiment. **e,** SETD6 does not methylate nucleosomes. Top: Autoradiogram of methylation reactions on recombinant nucleosomes or nucleosomes purified from HeLa cells with the indicated enzymes. Bottom: Coomassie stain of reactions is shown below the autoradiogram. Data represent duplicate experiments.

Supplementary Figure S2



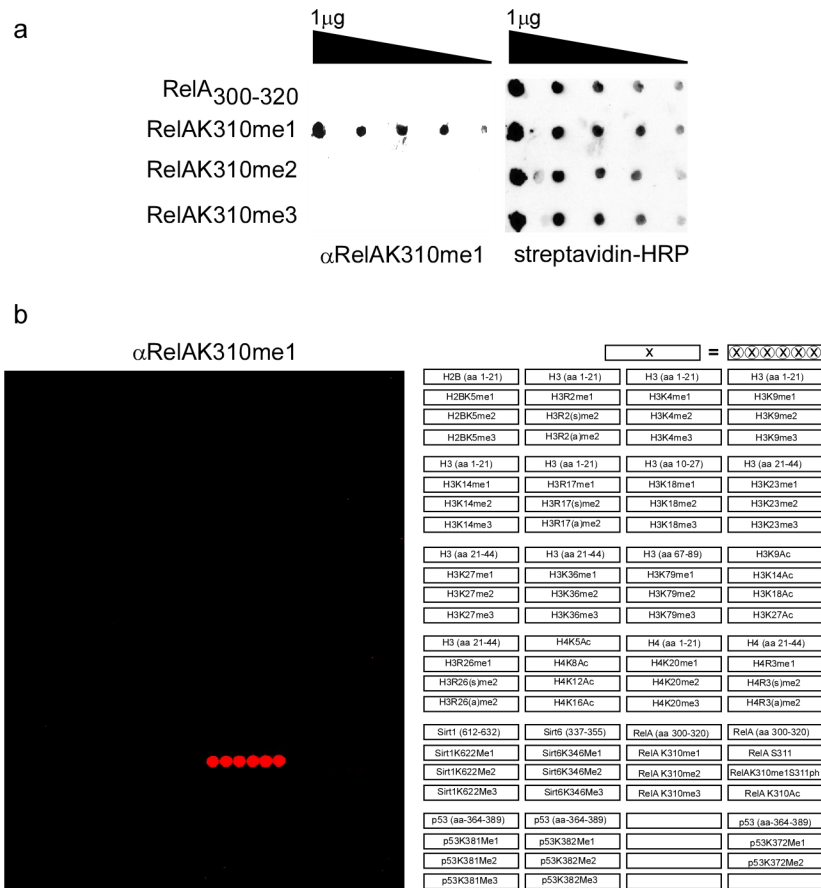
Supplementary Figure S2. SETD6 monomethylates RelA at K310. **a**, MS/MS spectra of SETD6-methylated RelA₃₀₀₋₃₂₀ peptide showing that RelAK310 is monomethylated. Underlined nominal fragment masses above and below the peptide sequence indicate positive annotation to the corresponding ions in the MS/MS spectra. Data shown for one of two biological replica. **b**, Dot blot analysis of RelA₃₀₀₋₃₂₀ biotinylated peptide alone or SETD6-methylated RelA₃₀₀₋₃₂₀ biotinylated peptide, using the indicated antibodies. Reactions were spotted at 1 ug/ul followed by 5X serial dilutions. HRP-conjugated streptavidin is shown as a loading control. Data represent duplicate experiments.

Supplementary Figure S3



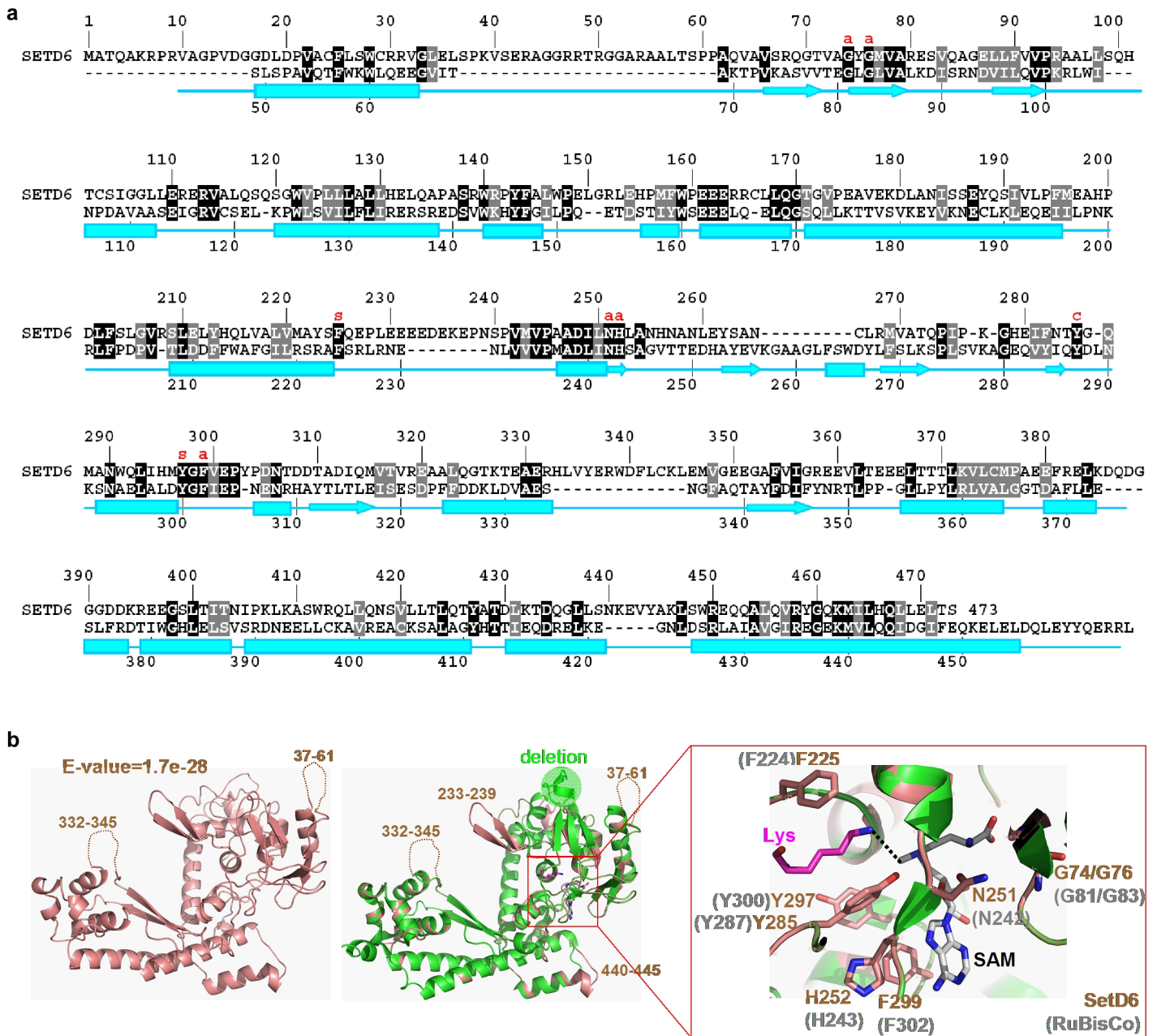
Supplementary Figure S3. Characterization of anti-SETD6 antibody and SETD6 cellular localization. **a**, α SETD6 antibody specifically recognizes overexpressed Flag-SETD6 but not several other PKMTs. Immunoblot analysis of the indicated Flag-tagged enzymes probed with either anti-Flag or anti-SETD6 as indicated. 1% of total WCE loaded in a single experiment. **b**, SETD6 is a nuclear protein. Immunofluorescence images of U2OS cells transfected with control or two independent SETD6 siRNAs. DAPI was used to stain nuclei as indicated. Data represent three independent biological replica.

Supplementary Figure S4



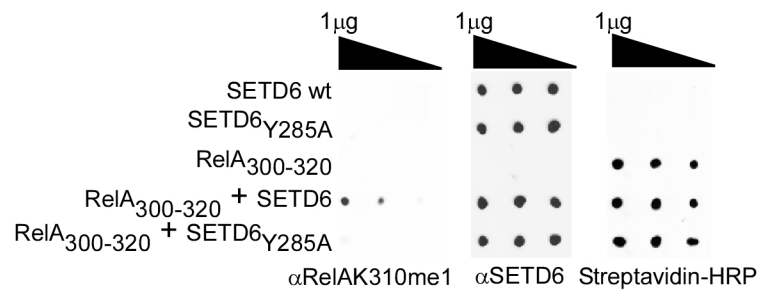
Supplementary Figure S4. Characterization of α RelAK310me1 epitope specificities. **a**, α RelAK310me1 antibody specifically recognizes the RelAK310me1 peptide. Dot blot analysis of the indicated biotinylated peptides and probed with α RelAK310me1 antibody. Peptides were spotted at 1 μ g/ μ l followed by 5X serial dilutions. HRP-conjugated streptavidin is shown as a loading control. Representative data from three independent biological replica **b**, α RelAK310me1 is highly specific for RelAK310me1 and does not recognize RelAK310me2, RelAK310me3 or other methylated or acetylated histone peptides. A peptide microarray containing the indicated peptides was probed with α RelAK310me1. A schematic of the peptides present on the slides and the order of spotting are shown to the right of the array.

Supplementary Figure S5



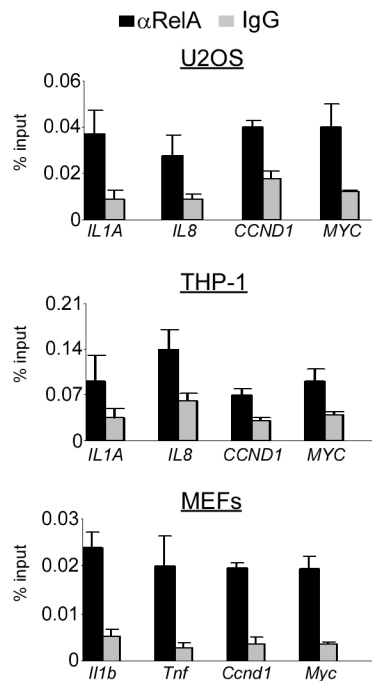
Supplementary Figure S5. Structure-based comparison of human SETD6 and Rubisco large-subunit methyltransferase (LSMT). **a**, Secondary structural elements (arrows for beta strands, and rectangles for alpha helices) are indicated for Rubisco methyltransferase (PDB 2H2E). White-on-black residues are invariant between the two sequences examined, while gray-highlighted positions are conserved (R and K; E and D; T and S; F and Y; V, I, L and M). Positions highlighted are responsible for various functions as indicated (a=AdoMet binding, s=substrate binding, c=catalysis). **b**, Homology modeling for SETD6 using the structure of Rubisco LSMT (<http://www.sbg.bio.ic.ac.uk/phyre>). After the manual adjustment of structure-based sequence alignment between SETD6 (right panel) and Rubisco LSMT (left panel), SETD6 has six insertions and one deletion. Two large insertions, residues 37-61 and 332-345, were not modeled. Two insertions, residues 233-239 and 332-345, are located on each side of the substrate binding cleft, whereas other insertions and the single deletion are located on the outer surface away from the substrate binding and the catalytic center. All residues identified in Rubisco LSMT as important for cofactor AdoMet binding, substrate binding, and catalysis are invariant in SETD6.

Supplementary Figure S6



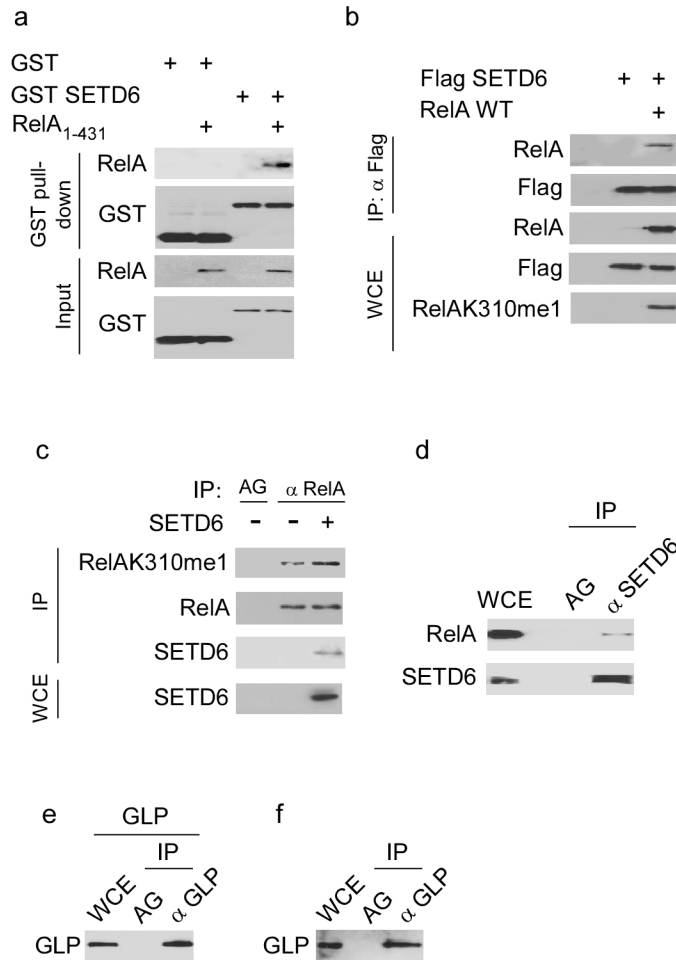
Supplementary Figure S6. Y285A in SETD6 substitution abrogates the catalytic activity of SETD6. Methylation reactions with SETD6 or SETD6_{Y285A} alone or with RelA₃₀₀₋₃₂₀ peptide were spotted and probed as indicated. Peptides were spotted at 1 ug/ul followed by 3X serial dilutions. HRP-conjugated streptavidin is shown as a loading control. Data representative of two independent experiments.

Supplementary Figure S7



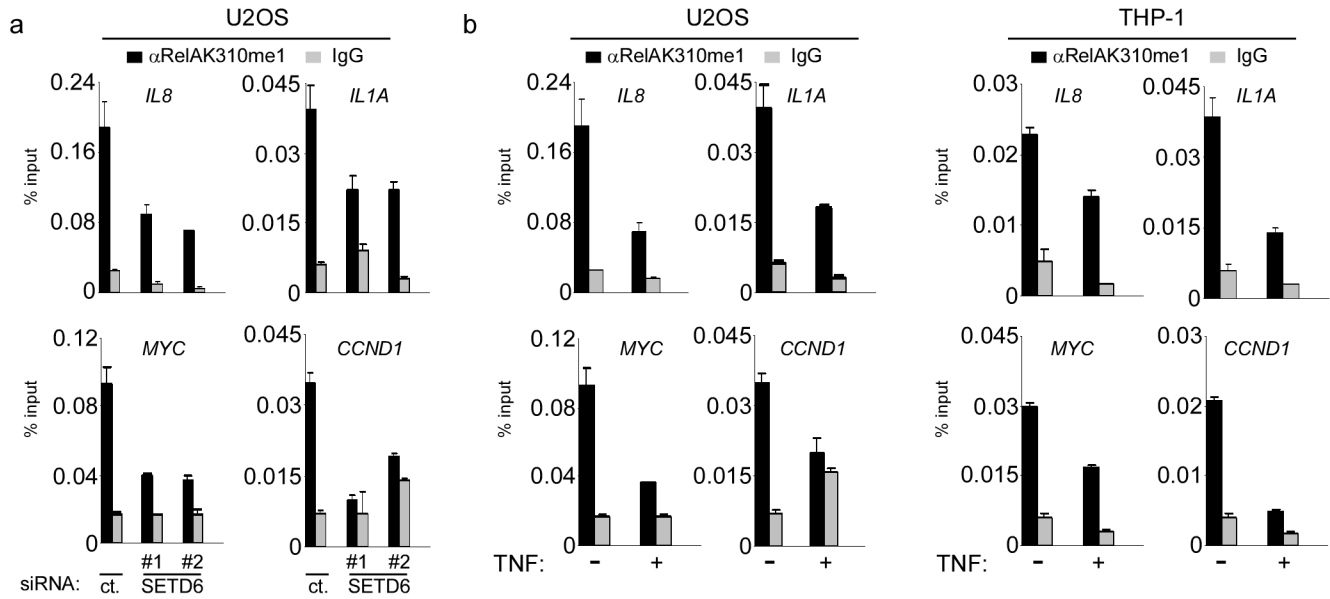
Supplementary Figure S7. RelA is enriched at chromatin in unstimulated cells. Occupancy of RelA (black bars) in U2OS, THP-1 and MEF cells at the indicated promoter was determined by real time (RT) PCR of ChIP samples. ChIP enrichment shown as % input (ChIP/input x 100). IgG (grey bars) was used as the ChIP negative control antibody. Error bars indicate \pm s.e.m. from at least three experiments.

Supplementary Figure S8



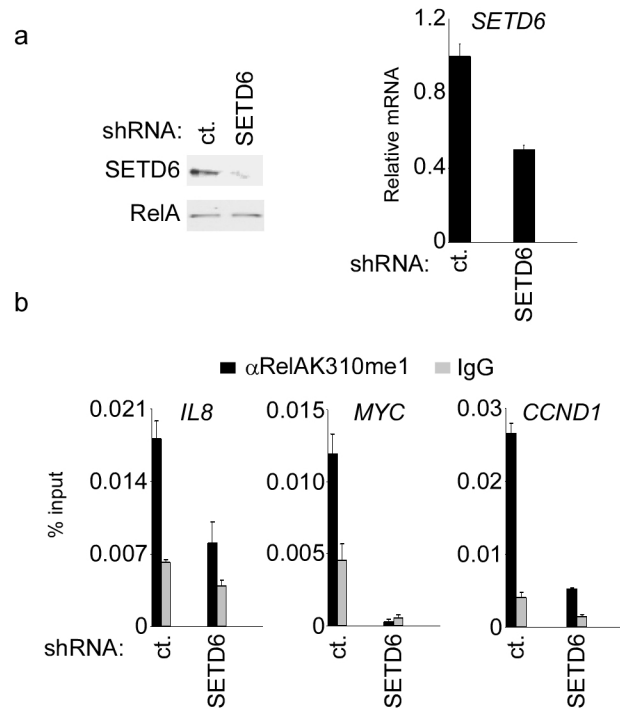
Supplementary Figure S8. RelA and SETD6 interact *in vitro* and in cells. **a**, SETD6 binds RelA *in vitro*. Immunoblot analysis of GST pull-down assays with GST alone or GST-SETD6 and recombinant RelA₁₋₄₃₁. Input: starting material used for pull down. Input represents 5% of total loaded. **b-d** SETD6 and RelA interact in cells. **b-c**, Immunoblot analysis with the indicated antibodies of (b) αFlag IPs or (c) αRelA IPs from 293T cells transfected with the indicated plasmids. 5% of total WCE is loaded. **d**, Endogenous SETD6 and RelA associate in cells. Immunoblot analysis of αSETD6 and control (Protein A/G beads) IPs from cell extracts probed with the indicated antibodies. 5% of total WCE loaded. Protein A/G beads (AG) were used as an antibody control in (c,d). Experiments in (b-d) each are representative of two independent biological replicas. **e-f** αGLP specifically immunoprecipitates GLP. Immunoblot analysis of αGLP and control (Protein A/G beads) IPs from 293T cells with transfected GLP (e) or endogenous GLP (f) probed with αGLP antibody. 5% and 10% of total WCE is loaded, respectively. Control experiments were performed once in (e) and (f).

Supplementary Figure S9



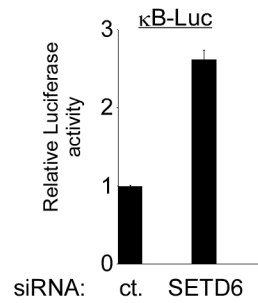
Supplementary Figure S9. Enrichment of RelAK310me1 at promoters of RelA target genes requires SETD6. The same ChIP experiment as shown in Figure 2a and 2b with IgG as a negative antibody control (grey bars). Error bars indicate \pm s.e.m. from at least three experiments.

Supplementary Figure S10



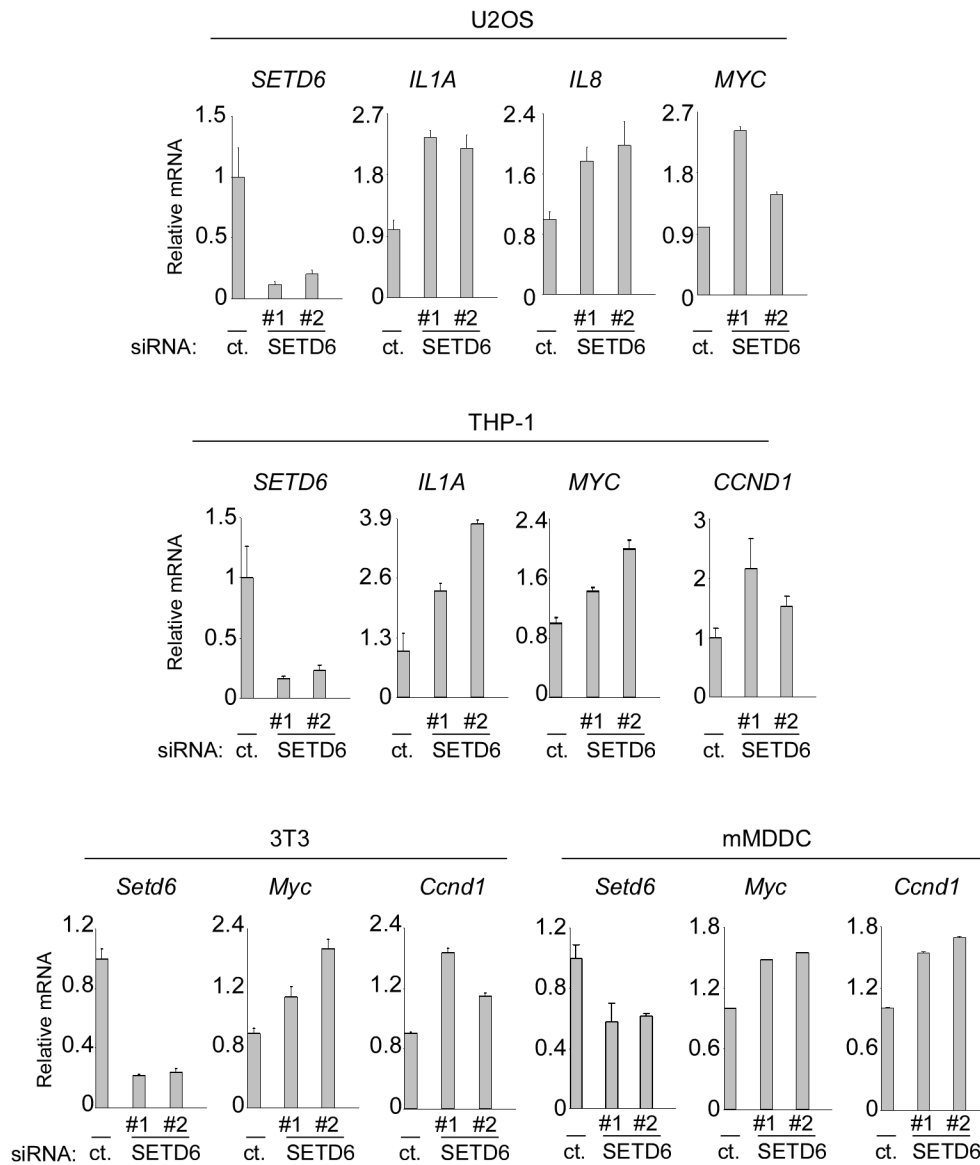
Supplementary Figure S10. Occupancy of RelAK310me1 at promoters of RelA target genes requires SETD6. **a**, Expression analysis of U2OS cells stably expressing control or SETD6 shRNA. Top left: Immunoblot analysis of indicated cell lines probed with α SETD6. RelA amounts are shown as loading control. Top right: real-time PCR of *SETD6* mRNA amounts in the indicated cell lines. Representative data from one of two independent experiments is shown. **b**, Presence of RelAK310me1 at RelA target genes requires SETD6. Occupancy of RelAK310me1 at the promoters of *IL8*, *MYC*, and *CCND1* (black bars) was determined by CHIP followed by real-time PCR. The enrichment of the CHIP is shown as % input. Error bars indicate \pm s.e.m. from at least three experiments. IgG was used for the CHIP negative antibody control (grey bars).

Supplementary Figure S11

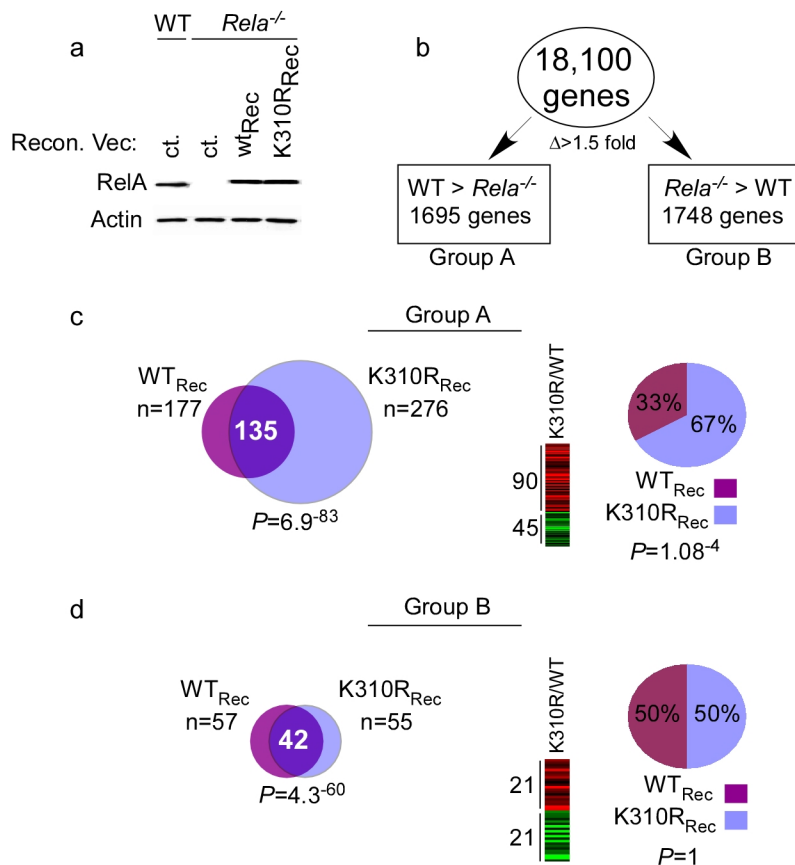


Supplementary Figure S11. SETD6 inhibits NF- κ B luciferase reporter activity. Depletion of SETD6 increases κ B-Luc reporter activity in unstimulated cells. κ B-Luc reporter activity normalized to Renilla was determined 24 hours after transfection of 293T cells with control or SETD6 siRNAs. Error bars indicate the s.e.m. from at least three experiments.

Supplementary Figure S12

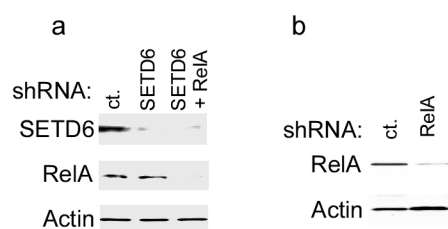


Supplementary Figure S12. SETD6 depletion increases RelA target gene expression in unstimulated cells. Real-time PCR analyses of the indicated mRNAs normalized to *gapdh* from U2OS, THP-1, 3T3 and primary mouse monocyte derived dendritic cells (mMDDC) transfected with control or two independent SETD6 siRNAs. Error bars indicate the s.e.m. from at least three experiments.



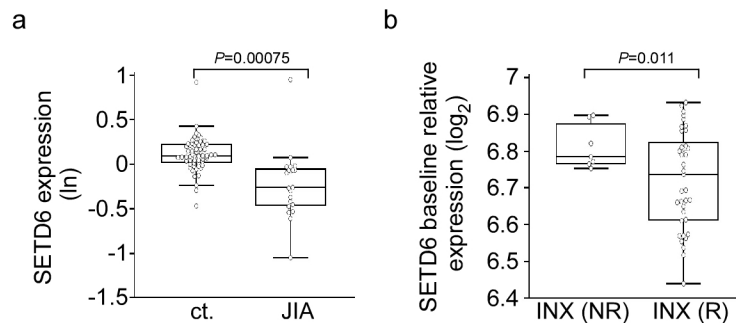
Supplementary Figure 13. Reconstitution of *RelA*^{-/-} MEFs with *RelA*_{K310R} leads to higher expression of *RelA*-dependent genes in the absence of stimulation than cells reconstituted with wild-type *RelA*. **a**, Mouse *RelA*^{-/-} fibroblast cells reconstituted with mouse *RelA* or *RelA*_{K310R} express ectopic *RelA* protein at amounts similar to those observed for endogenous *RelA* protein in *RelA*^{+/+} fibroblast cells. Immunoblot analysis of WCE from matched *RelA*^{+/+} or *RelA*^{-/-} 3T3 MEFs transduced with the indicated virus and probed with antibody against *RelA* or actin as a loading control. cont: empty pBABE-retro virus; wt_{REC}: *RelA* virus; K310R_{REC}: *RelA*_{K310R} virus. Representative data from one of two independent experiments is shown. **b**, Identification of *RelA*-dependent gene expression changes in MEFs. Whole-genome mRNA expression profiles under basal conditions were determined from *RelA*^{+/+} and *RelA*^{-/-} MEFs (from (a)) using Illumina-based mouse whole-genome expression arrays. 18,100 mouse target genes were clustered into two groups that showed a $\Delta > \pm 1.5$ in expression between *RelA*^{+/+} and *RelA*^{-/-} MEFs. Group A (wt > *RelA*^{-/-}): 1695 genes that were highly expressed in *RelA*^{+/+} relative to *RelA*^{-/-} cells. Group B (*RelA*^{-/-} > wt): 1748 genes that were highly expressed in the *RelA*^{-/-} cells relative to wt cells. **c**, Reconstitution of *RelA*^{-/-} cells with *RelA*_{K310R} (K310R_{REC}) leads to higher expression of Group A genes than reconstitution with wild-type *RelA* (wt_{REC}). Left: Venn diagrams showing genes that are upregulated > 1.5 fold in *RelA* and *RelA*_{K310R} reconstituted cells relative to *RelA*^{-/-} cells and present in the Group A category described in (b). Note the greater number of genes in K310R_{REC} (276) versus wt_{REC} (177) and that 135 of the genes are present in both reconstitutions. Right: Heat map comparing log₂ transformed expression abundance of the 135 overlapping genes between K310R_{REC} and wt_{REC}. The pie chart shows that 67% have higher expression in K310R_{REC} versus wt_{REC}. Heat map: Red and green represent up- and down-regulated genes, respectively. p-values for the Venn diagram and pie charts were calculated using Fisher's Exact Test and Chi-Square analysis, respectively. Mean values from two biological replicates (independently isolated RNA) were used in all analyses. **d**, K310R_{REC} and wt_{REC} expression changes are similar in Group B genes. Analysis as in (c) on group B genes. No significant difference in down-regulated genes was observed between the two reconstituted cell lines, suggesting that K310 plays a specific role in upregulating *RelA*-dependent genes.

Supplementary Figure S14



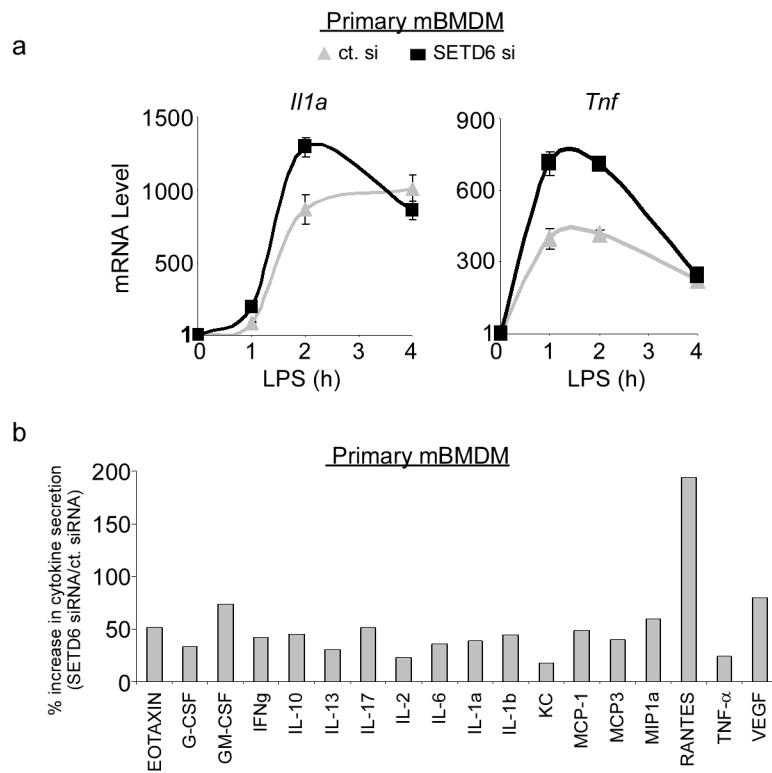
Supplementary Figure S14. Knockdown efficiency of U2OS stable cell lines. a-b, Immunoblot analysis of WCE from SETD6 knockdown, SETD6/RelA double knockdown (a) and RelA knockdown (b) of U2OS stable cell lines used in Figure 3a-b, probed with the indicated antibodies. Representative data from one of two independent experiments is shown for a and b.

Supplementary Figure S15



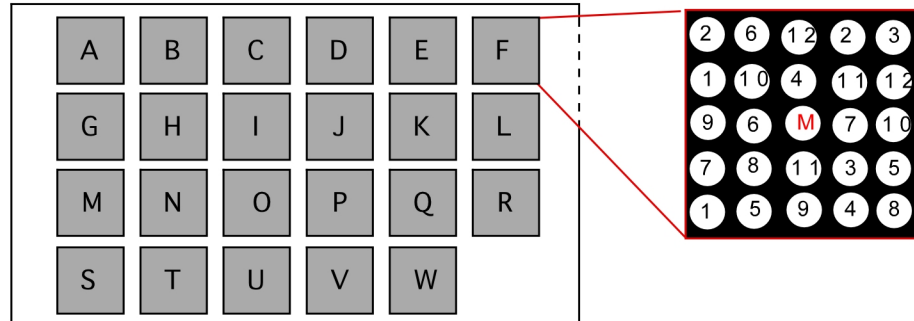
Supplementary Figure S15. SETD6 expression level in inflammatory diseases. a, SETD6 expression level is lower ($p = 0.00075$, two-tailed t test) in systemic juvenile idiopathic arthritis (JIA) ($n = 21$) compared to healthy controls ($n = 59$). Expression level is shown as the natural log of median normalized expression values of the sample. Data retrieved from GEO accession GSE13501 and GSE13849. **b,** SETD6 expression level is lower ($p = 0.011$, two-tailed t test) in infliximab responders (INX(R), $n = 37$) compared to non-responders (INX(NR), $n = 7$) in rheumatoid arthritis (RA) patients. Expression level is shown as the \log_2 normalized baseline relative expression values of the sample. Data retrieved from GEO accession GDS3628.

Supplementary Figure S16



Supplementary Figure S16. a, SETD6 depletion in primary mouse BMDM cells increases production of RelA target gene mRNA and protein. Primary mouse BMDM (mBMDM) cells were transfected with control or SETD6 siRNAs and treated \pm LPS for the indicated time points, and the amount of the indicated mRNAs were measured by real-time PCR analysis. Error bars indicate the s.e.m. from at least three experiments. b, SETD6 depletion enhances the secretion of a panel of RelA-dependent cytokines in response to LPS. Primary mBMDM cells were transfected with a control or SETD6 siRNA and treated \pm LPS for 2 hours. Secretion of the indicated cytokines into the culture supernatant was measured by multiplex cytokine assay. Results are presented as % increase in cytokine amount in SETD6-depleted cells compared to control cells. Representative data from two independent experiments repeated in duplicate is shown.

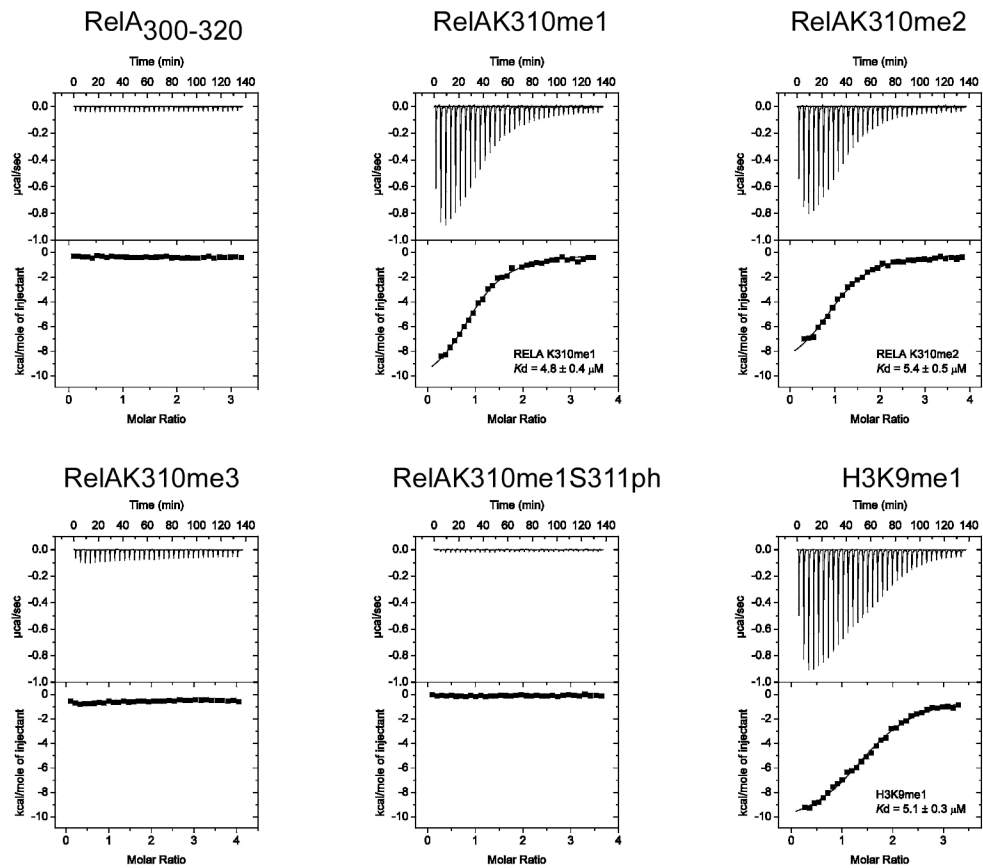
Supplementary Figure S17



<p>TUDOR</p> <p>A1 TDRD1(1)(NP_112568)</p> <p>A2 TDRD1(2)(NP_112568)</p> <p>A3 TDRD2(NP_006853)</p> <p>A4 TDRD3(Q9H7E2)</p> <p>A5 TDRD4-1(Q9NUY9)</p> <p>A6 TDRD4-2(Q9NUY9)</p> <p>A7 TDRD4-3(Q9NUY9)</p> <p>A8 TDRD5(Q8NAT2)</p> <p>A9 TDRD7-1(NP_055105)</p> <p>A10 TDRD7-3(NP_055105)</p> <p>A11 TDRD7(1-3)(NP_055105)</p> <p>A12 Tudor 9(NP_694591)</p>	<p>TUDOR</p> <p>B1 EBNA-2Co-A(NP_055205)</p> <p>B2 Ret-bp1(AA828543)</p> <p>B3 M96(AAH10013)</p> <p>B4 STK31(Q9BXU1)</p> <p>B5 53BP1(1-2)(NP_005648)</p> <p>B6 53BP1(1)(NP_005648)</p> <p>B7 53BP1(2)(NP_005648)</p> <p>B8 Anchor (NP_003479)</p> <p>B9 2B(NP_055830)</p> <p>B10 2C(NP_055876)</p> <p>B11 RBP1 like-2 (NP_112739)</p> <p>B12 SMN (NP_075012)</p>	<p>TUDOR</p> <p>C1 ESET(NP_036564)</p> <p>C2 CGI-72(NP_057102)</p> <p>C3 FX(AAH67272)</p> <p>C4 PHF20(NP_057520)</p> <p>C5 Pombe 1(CAA22823)</p> <p>C6 Pombe 2(CAB39904)</p> <p>C7 Colon 1(AAC18034)</p> <p>C8 Colon 2(AAC18034)</p> <p>C9 Colon 2(AAC18034)</p> <p>C10 JMJD2A-1(NP_055478)</p> <p>C11 JMJD2A-2(NP_055478)</p> <p>C12 JMJD2A1-2(NP_055478)</p>	<p>TUDOR</p> <p>D1 Lin9 TDR(b)(AAH65302)</p> <p>D2 LBR TDR(NP_919424)</p> <p>D3 LBR211(NP_919424)</p> <p>D4 SPF30(c)(O75940)</p> <p>D5 JMN 2B WT(F)(NP_055830)</p> <p>D6 Lin9 DIRP(b)(AAH65302)</p> <p>D7 Colon Short 1-2(AAC18034)</p> <p>D8 ARH4 (e)(NM_002892)</p> <p>D9 SETB1 (e)(NM_012432)</p> <p>D10 SND1 (e)(NM_014390)</p> <p>D11 STK31(e) (NM_031414)</p> <p>D12 TDR Like Spindlin1</p>	<p>MBT-TUDOR</p> <p>E1 L(3)MBT(NP_056293)</p> <p>E2 SCML1(NP_057413)</p> <p>E3 SCML2(AAH64617)</p> <p>E4 SCMH1(AAH21252)</p> <p>E5 LML2(Q969R5)</p> <p>E6 KIAA1617(XP_166140)</p> <p>E7 PHF20 MBT(NP_057520)</p> <p>E8 CG1-72-MBT(NP_057102)</p> <p>E9 PHF20 MBT+TDR(NP_057520)</p> <p>E10 dSfmbt HIS(a)(NP_609606)</p> <p>E11 L(3)MBT1-3(NP_056293)</p> <p>E12 MBT SFMBT2(1-4)(AB046837)</p>	<p>WD40</p> <p>F1 WDR5(d)(NP_543124)</p> <p>F2 WDR9(NM_018963)</p> <p>F3 TBLR12(d)(CAA73319)</p> <p>F4 TBLR1(d)(AF314544)</p> <p>F5 RbAb46(d)(BT007309)</p> <p>F6 RbAb46(d)(X74262)</p> <p>F7 EED(NM_152991)</p> <p>F8 HIRA(CR456503)</p> <p>F9 WDHD1(NM_007086)</p> <p>F10 Mep50(d)(AF478464)</p> <p>F11 DDB2(NM_000107)</p> <p>F12 BRWD3(NM_153252)</p>
<p>PhD</p> <p>G1 BPTF(P+B)(BAA89208)</p> <p>G2 ING2(e)(AAH50003)</p> <p>G3 PHF2(NP_005383)</p> <p>G4 PHF8(CA142860)</p> <p>G5 DATF1(CA195708)</p> <p>G6 Rag2(NM_000536)</p> <p>G7 PCCX1(NP_055408)</p> <p>G8 P300(P+B)(NM_001429)</p> <p>G9 PHF20 PHD(NP_057520)</p> <p>G10 PHD PHF3</p> <p>G11 PHD PHF5</p> <p>G12 PHD CHD5 (1-2)</p>	<p>PhD</p> <p>H1 Dnmt3a-His/GST(g)</p> <p>H2 Dnmt3b-His/GST(g)</p> <p>H3 DnMT3L N-term-His/GST(g)</p> <p>H4 Trim24 Rad-PhD (u)</p> <p>H5 ING3</p> <p>H6 ING4</p> <p>H7 ING5</p> <p>H8 PHD_TIF1A(e) (O15164)</p> <p>H9 TRIM66(e) (O15016)</p> <p>H10 BRPF1(e) (P55201)</p> <p>H11 MLL4(e) (Q9UMN6)</p> <p>H12 MTF2(e) (Q9Y483)</p>	<p>PhD +</p> <p>I1 JMJD2APhd+2Tudor(NP_055478)</p> <p>I2 JMJCPhd(NP_055478)</p> <p>I3 M96Tudor+Phd(AAH10013)</p> <p>I4 MYST4Phd+Phd(AAH48199)</p> <p>I5 NSD1Phd+PWWP(Q96L73)</p> <p>I6 WHKCB1Phd+PWWP(NP_579877)</p> <p>I7 PRKCB1Phd+BRD(AAH12586)</p> <p>I8 BS69Phd+ BRD(AAH12586)</p> <p>I9 ATRX</p> <p>I10 RAL1</p> <p>I11 BAZ1b/WSTF</p> <p>I12 CBP</p>	<p>BROMO</p> <p>J1 GCN5(Q92830)</p> <p>J2 TAF1- D1(NP_620278)</p> <p>J3 TAF1- D2(NP_620278)</p> <p>J4 P/CAF (S71788)</p> <p>J5 SP140(NP_009168)</p> <p>J6 SMF2 beta(S45252)</p> <p>J7 SMAP(NP_899203)</p> <p>J8 BAF180 1-2(NP_060635)</p> <p>J9 BAF180-3(NP_060635)</p> <p>J10 BAF180 3-4(NP_060635)</p> <p>J11 BAF180 5-6(NP_060635)</p>	<p>BROMO</p> <p>K1 TIF1 alpha(AAD17258)</p> <p>K2 KAP-1(AAB37341)</p> <p>K3 P300(NP_004371)</p> <p>K4 WDR9 1-2(Q9NSI6)</p> <p>K5 WDR9 1(Q9NSI6)</p> <p>K6 WDR9 2(Q9NSI6)</p> <p>K7 BAZ(NP_075381)</p> <p>K8 BRD1 1-2(AAH62700)</p> <p>K9 BRD1 1(AAH62700)</p> <p>K10 BRD1 2(AAH62700)</p> <p>K11 BRD4 1SANT/SWIRM</p>	<p>SANT / TSN</p> <p>L1 MPP11-like(XP_379909)</p> <p>L2 MTA1(Q13330)</p> <p>L3 N-CoR2(Q9Y618)</p> <p>L4 N-CoR2-1(Q9Y618)</p> <p>L5 N-CoR2-2(Q9Y618)</p> <p>L6 N-CoR2(NP_006302)</p> <p>L7 RERE(AAH62342)</p> <p>L8 ADA2-SANT(NP_001479)</p> <p>L9 Zustin Rel.(XP_168590)</p> <p>L10 TSN (t)</p> <p>L11 TSN m5(t)</p> <p>L12 TSN m6(t)</p>
<p>CHROMO</p> <p>M1 TIP60(h)(AAB18236)</p> <p>M2 CHD2(h)(AAB87382)</p> <p>M3 CHD4(h)(AAH38596)</p> <p>M4 MPP8(h)</p> <p>M5 SMARCC2(h)(AAH26222)</p> <p>M6 MRG1 5(h)(AAD29872)</p> <p>M7 BRP1(h)(AAD41239)</p> <p>M8 PC2(h)(AAB80718)</p> <p>M9 PC3(h)(AAG09180)</p> <p>M10 CHD5(h)(AAK56405)</p> <p>M11 CHD7 (1-2)(AB037837)</p> <p>M12 CBX6/NPCD(BC012111)</p>	<p>CHROMO</p> <p>N1 Mi-2(h)(CAA60384)</p> <p>N2 HP1 alpha(h)(P45973)</p> <p>N3 HP1 gamma(h)(NP_057671)</p> <p>N4 Msi3-like(h)(AAD38499)</p> <p>N5 SUV39H1(h)(AAB92224)</p> <p>N6 CBX1(h)(AAD21972)</p> <p>N7 HP1 beta(h)(P23197)</p> <p>N8 CDY1(h)(AAD22735)</p> <p>N9 CHD1(e)</p> <p>N10 CBX4(e) (NM_003655)</p> <p>N11 CBX7(e) (NM_175709)</p> <p>N12 CBX5(e) (NM_012117)</p>	<p>CHROMO / BRK / MRG</p> <p>O1 CBX3(e) (NM_016587)</p> <p>O2 CBX2(e) (NM_005189)</p> <p>O3 CDYL2(e) (NM_152342)</p> <p>O4 CBX8(e) (NM_020649)</p> <p>O5 BRK_SMC2A (e)(NM_003070)</p> <p>O6 BRK_SMC4(e) (NM_003072)</p> <p>O7 BRK_CHD6(e) (NM_032221)</p> <p>O8 BRK_CHD7 (e)(NM_017780)</p> <p>O9 BRK_Q6D7K9 (e)(NM_025134)</p> <p>O10 MRG_M03L1(e) (NM_078629)</p> <p>O11 MRG_M04L1(e) (NM_206839)</p>	<p>PWWP</p> <p>P1 BRPF1(AAH53851)</p> <p>P2 BS69(AAH12586)</p> <p>P3 DNMT3B(Q9UBC3)</p> <p>P4 HDGF (P51858)</p> <p>P5 HRP-3(BAA90477)</p> <p>P6 MSH6(P52701)</p> <p>P7 NSD1(Q96L73)</p> <p>P8 WHSC1(NP_579877)</p> <p>P9 PSIP1(e) (NM_033222)</p> <p>P10 BRD1(e) (NM_014577)</p> <p>P11 ZCWP1 (e)(AL1136735)</p> <p>P12 MBD5 (e)(NM_018328)</p>	<p>PWWP / CW</p> <p>Q1 PWWP-PKCB1(e) (NM_183047)</p> <p>Q2 PWWP-HDGR3(e) (NM_016073)</p> <p>Q3 PWWP-DNM3A (e) (NM_175629)</p> <p>Q4 CW1(BAA74875)</p> <p>Q5 CW3(AAH02725)</p> <p>Q6 CW4(CAD23056)</p> <p>Q7 CW5(BAA09485)</p> <p>Q8 CW6(XP_087384)</p> <p>Q9 FATM14</p> <p>Q10 TAF10 TAFH</p> <p>Q11 TULP1</p>	<p>ANK</p> <p>R1 BARD1 (NP_000456)</p> <p>R2 G9a delta (CAA49491)</p> <p>R3 hG9a#3(CAA49491)</p> <p>R4 HEUHM1</p> <p>R5 mG9a</p> <p>R6 MMRP (NP_059990)</p> <p>R7 RFX delta(CAG3262)</p> <p>R8 RFX#3(CAG3262)</p> <p>R9 Ancol1(AA545544)</p> <p>R10 53BP2(AAH58918)</p> <p>R11 Notch (NP_060087)</p>
<p>FHA/KH/BRCT(i)</p> <p>S1 RAD 53 FHA2(j)</p> <p>S2 RAD 53 FHA1(j)</p> <p>S3 MDC1 FHA (i)</p> <p>S4 KI FHA (1-168)(k)</p> <p>S5 CHK2 FHA</p> <p>S6 SAM 68 KH(i)</p> <p>S7 QKI KH(i)</p> <p>S8 BRCT FCP1</p> <p>S9 BRCT Bard1</p> <p>S10 BRCT TDT</p>	<p>BRCT(i)</p> <p>T1 BRCA1</p> <p>T2 53BP1</p> <p>T3 Ctb2</p> <p>T4 TopBP1-6</p> <p>T5 Rad4 I, II</p> <p>T6 Rad4 III, IV</p> <p>T7 Ect2</p> <p>T8 LigaseV</p> <p>T9 MDC1</p> <p>T10 Rad9</p> <p>T11 REV1</p> <p>T12 DNA LIGASE II</p>	<p>Others</p> <p>U1 NO66 M1 (p)</p> <p>U2 NO66M2 (p)</p> <p>U3 NO66 M5 (p)</p> <p>U4 NO66 JMJC (p)</p> <p>U5 His-DnaA 230 (q)</p> <p>U6 His-DnaA 343 (q)</p> <p>U7 SWIRM_KIAA1915(BAB67808)</p> <p>U8 SWIRM_KIAA0601(CAB72299)</p> <p>U9 SWIRM_ADA2(NP_001479)</p> <p>U10 SWIRM_SMR2(e) (NM_033222)</p> <p>U11 SWIRM_SMR1(e) (NM_014577)</p>	<p>Other</p> <p>V1 POZ Zbtb4(m)</p> <p>V2 POZ KA150(m)</p> <p>V3 His-MBD1(m)</p> <p>V4 MBD2(n)</p> <p>V5 His-MBD3(n)</p> <p>V6 MeCP2(n)</p> <p>V7 SFMBT F.L(o)</p> <p>V8 SFMBT 4xMBT(o)</p> <p>V9 WD40 Tap5</p> <p>V10 WD40 CSA</p> <p>V11 Shooting (r)</p> <p>V12 PHD_ZFP-1(s)</p>	<p>Others</p> <p>W1 FG_Nup 116(y)</p> <p>W2 FG_Nup 42(y)</p> <p>W3 FG_Nup2(y)</p> <p>W4 XP 120(x)</p> <p>W5 X Arm ARV(x)</p> <p>W6 X Arm Beta(x)</p> <p>W7 X Arm Delta(x)</p> <p>W8 Lap2 Lem (v)</p> <p>W9 Lap2 Lem Mut(v)</p> <p>W10 Lap2 Lem-like7(v)</p> <p>W11 Kim17 -His/GST(w)</p> <p>W12 Plantagenet_FXR1(e)(NM_005087)</p>	

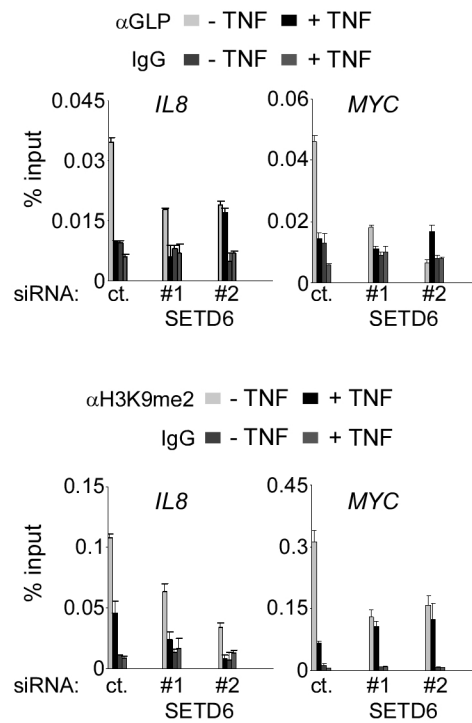
Supplementary Figure S17. Schematic representation of CADOR (chromatin-associated domain array) microarrays. The indicated collection of 268 GST fusion proteins printed in duplicate on a nitrocellulose slide is shown.

Supplementary Figure S18



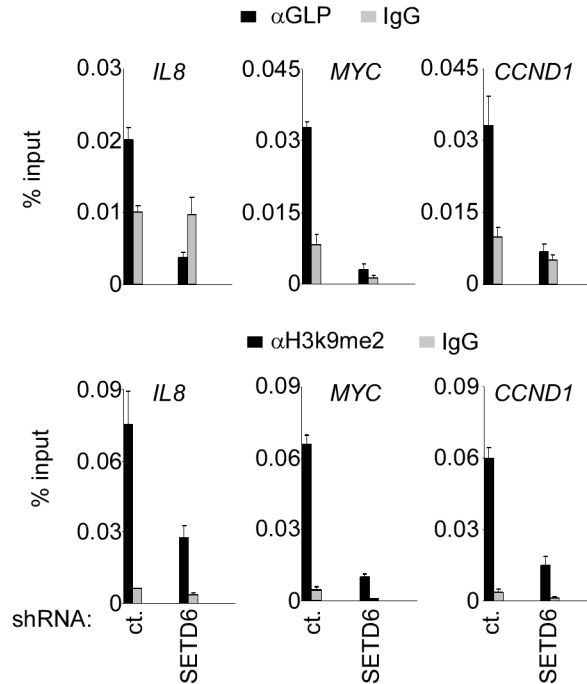
Isothermal calorimetry (ITC) traces for GLP_{ANK} binding experiments. The dissociation constants (K_D) are given in Figure 4c. Peptides used are indicated. Representative data of three independent experiments is shown.

Supplementary Figure S19



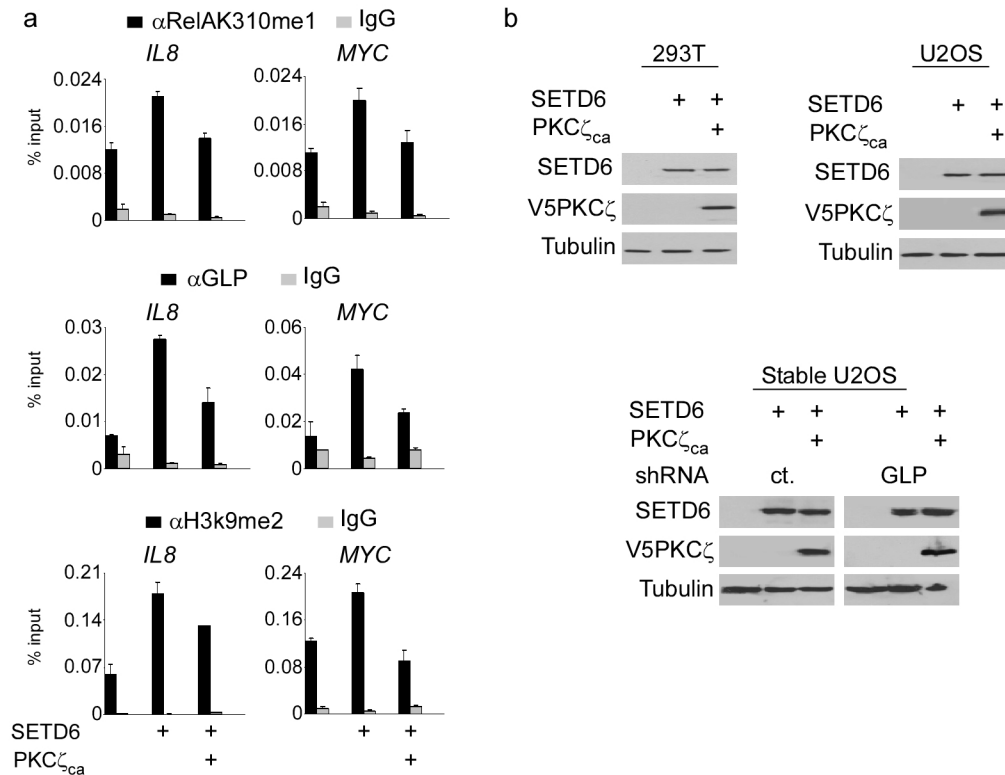
Supplementary Figure S19. GLP and H3K9me2 occupancy at promoters of RelA target genes requires SETD6 and is inhibited by TNF treatment. The same ChIP experiment as shown in Figure 5h with IgG as a negative antibody control (grey bars). Error bars indicate \pm s.e.m. from at least three experiments.

Supplementary Figure S20



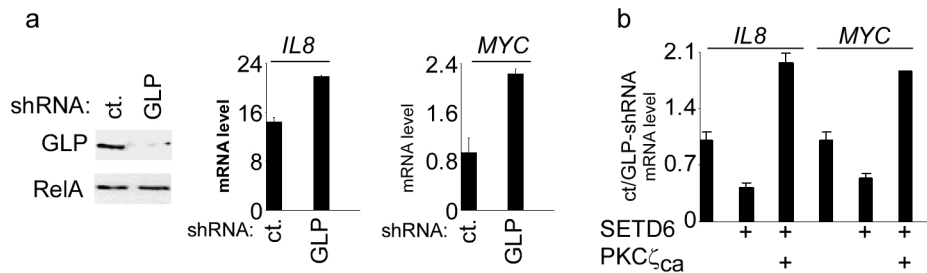
Supplementary Figure S20. GLP and H3K9me2 occupancy at RelA target gene promoters requires SETD6. Occupancy of GLP and H3K9me2 in U2OS cells stably expressing control or SETD6 shRNA at the promoters of *IL8*, *MYC* and *CCND1* (black bars) was determined by real-time PCR of ChIP samples. The enrichment of the ChIP is shown as % input. Error bars indicate the s.e.m. from at least three experiments. IgG was used as the ChIP negative antibody control (grey bars).

Supplementary Figure S21



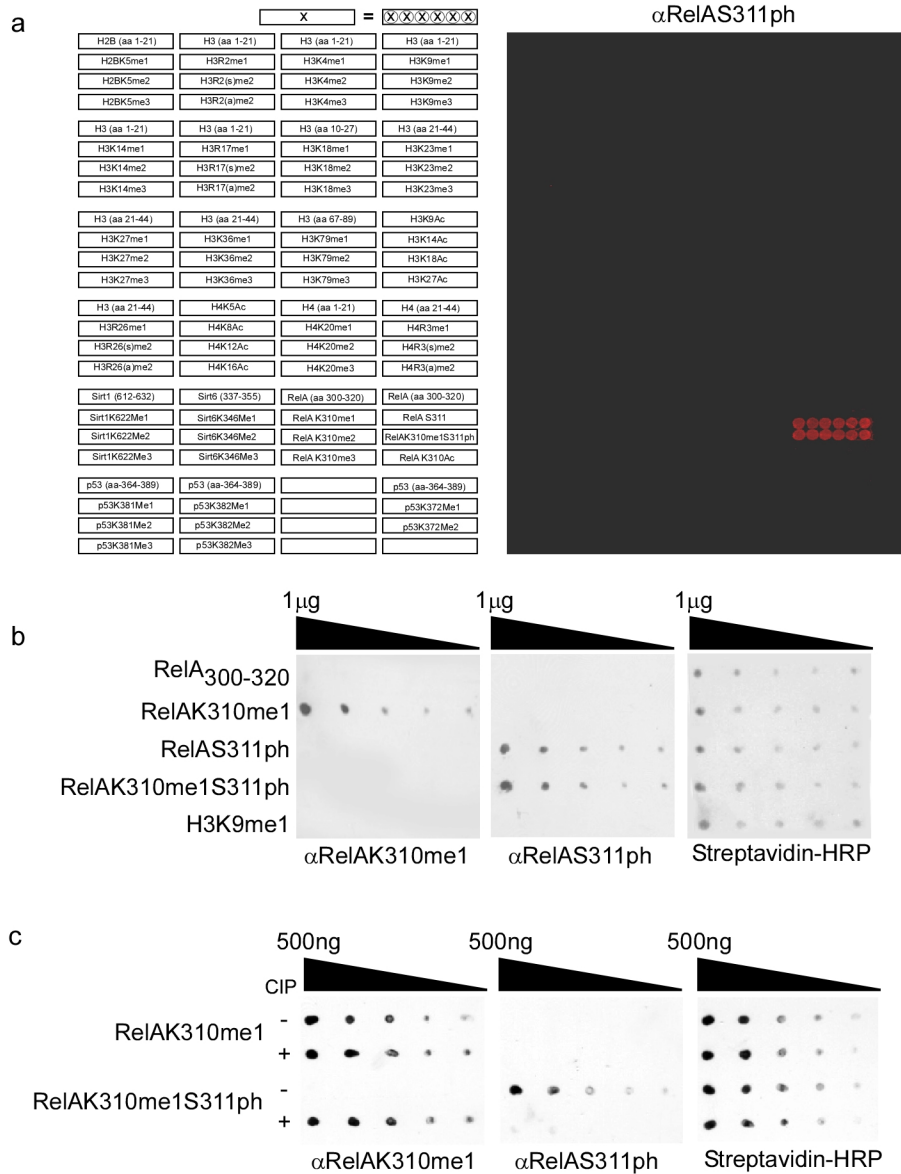
Supplementary Figure S21. SETD6-driven increases in GLP and H3K9me2 occupancy at the *IL8* and *MYC* promoters are antagonized by PKC ζ_{ca} . **a**, Occupancy of RelAK310me1, GLP, and H3K9me2 at the *IL8* and *MYC* promoters in U2OS cells transfected with the indicated plasmids was determined by real-time PCR of ChIP samples (black bars). The enrichment of the ChIP is shown as % input. Error bars indicate the s.e.m. from at least three experiments. IgG was used as the ChIP negative antibody control (grey bars). **b**, Immunoblot analysis of SETD6 and PKC ζ_{ca} protein amount in transfected cells. Immunoblot analysis with the indicated antibodies of WCE from 293T cells, U2OS cells, and U2OS cells stably expressing control or GLP shRNA, that have been transfected with the indicated plasmids. Tubulin is shown as a loading control.

Supplementary Figure S22



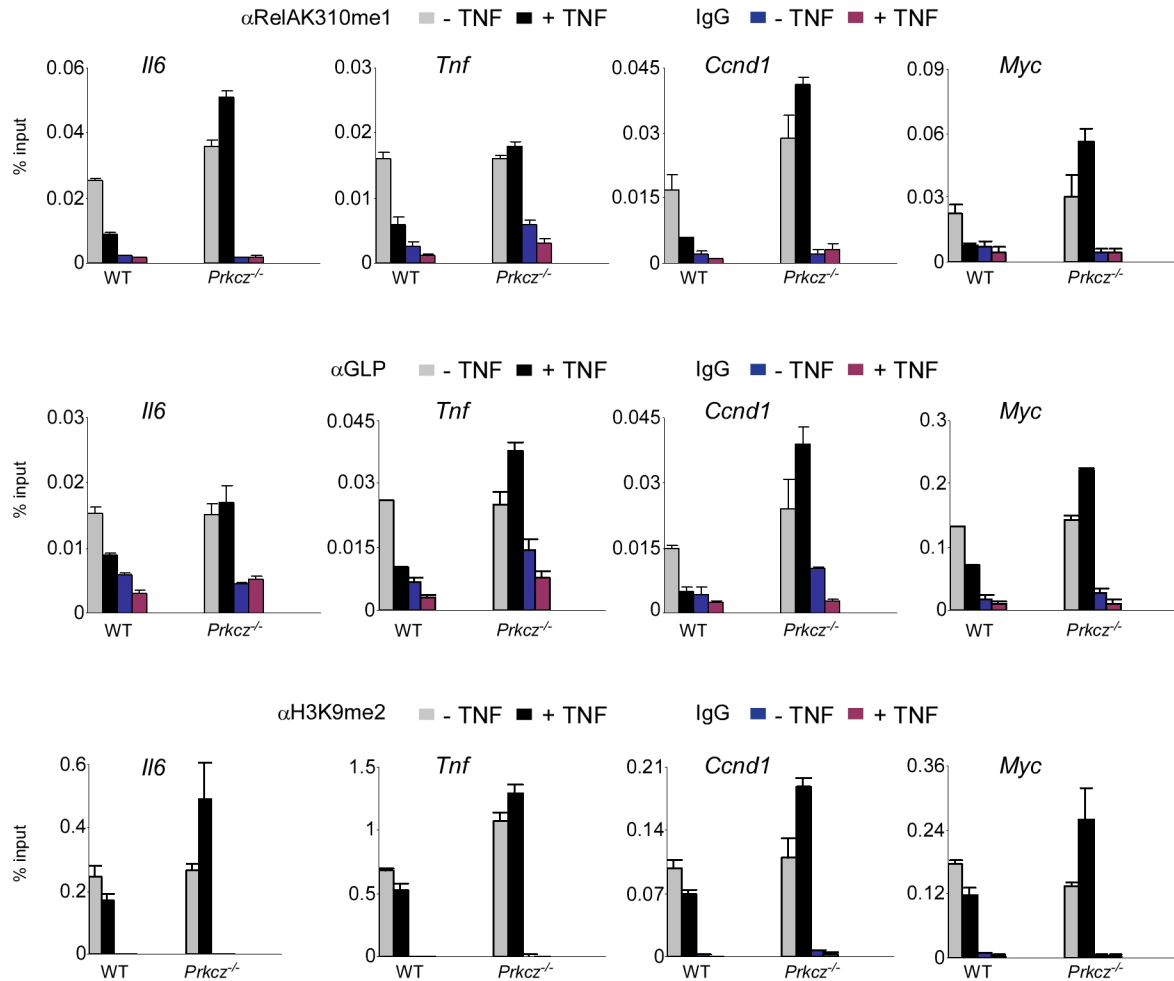
Supplementary Figure S22. PKC ζ antagonism of SETD6 is abrogated in GLP-depleted cells. **a**, GLP depletion induces TNF-dependent upregulation of *IL8* and *myc* mRNAs. Left: Immunoblot analysis of U2OS WCEs treated with control or GLP shRNA and probed with α GLP. RelA abundance is shown as a loading control. Right: Expression of indicated mRNAs in U2OS cells stably expressing control or GLP shRNA and treated with TNF (10 ng/ml) was determined by RT PCR. **b**, SETD6-driven repression of *IL8* and *myc* expression requires GLP and is antagonized by co-expression of PKC ζ_{ca} . Control and GLP stable knockdown U2OS cells as in (a) were transfected with the indicated plasmids and *IL8* and *myc* mRNA amounts were determined as described in (a). y-axis: expression of normalized mRNA amounts shown as fold change of control shRNA/GLP shRNA. Error bars indicate \pm s.e.m. from at least three experiments.

Supplementary Figure S23



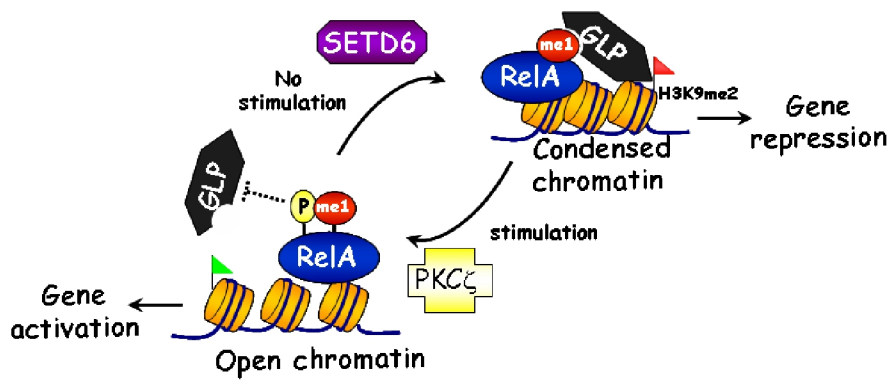
Supplementary Figure S23. Phosphorylation of RelA at S311 blocks the recognition of the RelAK310me1 epitope. a, α RelAS311ph recognizes S311 phosphorylation irrespective of K310 monomethylation status. A peptide microarray containing the indicated peptides was probed with α RelAS311ph antibody. Left: A schematic of the peptides present on the slides and the order of spotting. **b**, Dot blot analysis of the indicated biotinylated peptides, probed with the indicated antibodies. Peptides were spotted at 1 μ g/ μ l followed by 5X serial dilutions. HRP-conjugated streptavidin is shown as a loading control. Representative data of two independent experiments is shown. **c**, Dot blot analysis of the indicated biotinylated peptides \pm calf intestinal alkaline phosphatase (CIP) treatment for 1 hr, probed with the indicated antibodies. Peptides were spotted at 0.5 μ g/ μ l followed by 5X serial dilutions. HRP-conjugated streptavidin is shown as a loading control.

Supplementary Figure S24



Supplementary Figure S24. Requirement for PKC ζ to decrease occupancy of RelAK310me1, GLP, and H3K9me2 at chromatin of RelA target gene promoters in response to NF- κ B stimulation. The same ChIP experiment as shown in Figure 7a with IgG as a negative antibody control. Error bars indicate \pm s.e.m. from at least three experiments.

Supplementary Figure S25



Supplementary Figure S25. Model of the interplay between SETD6, RelA, GLP, and PKC ζ in the regulation of NF- κ B signaling (see text).