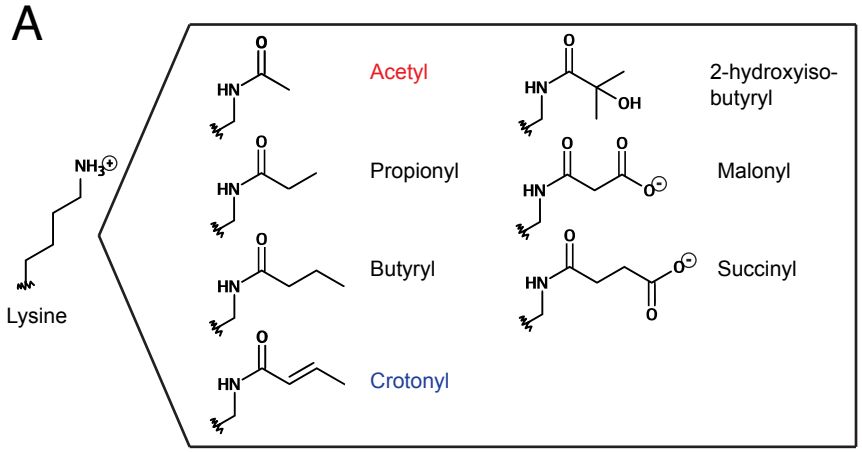
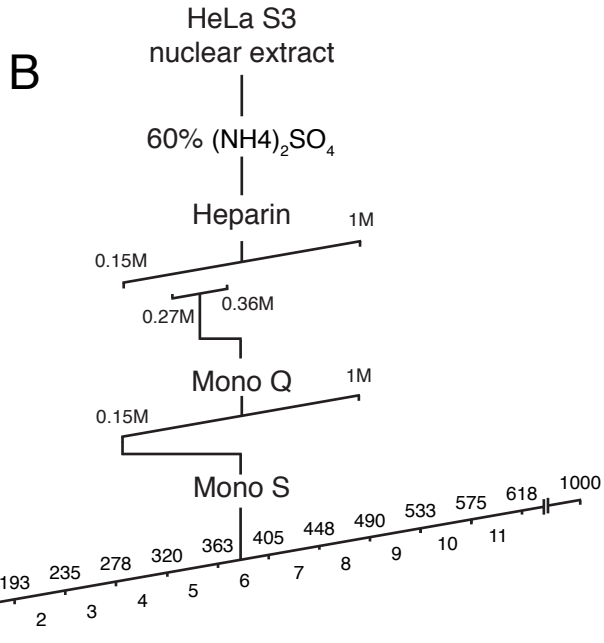
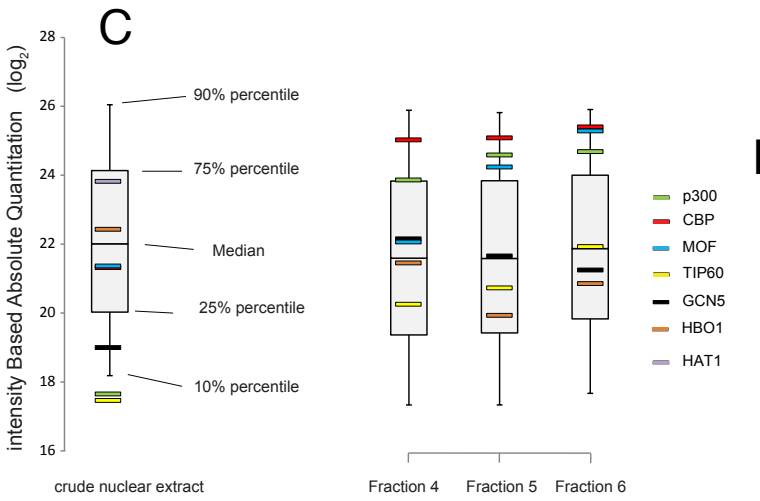
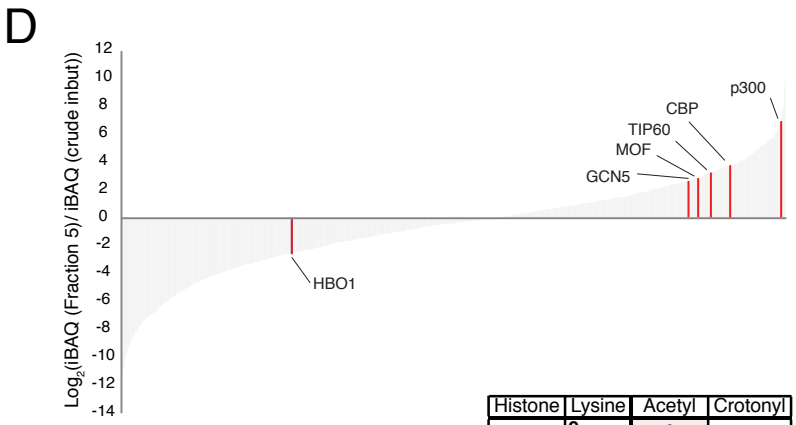
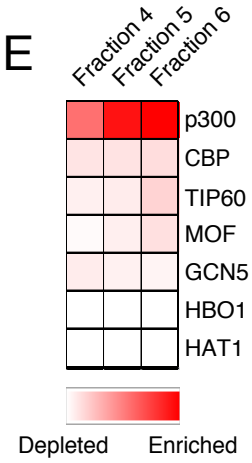
Figure S1 (related to Figure 1)

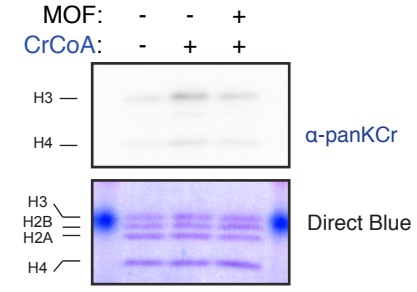


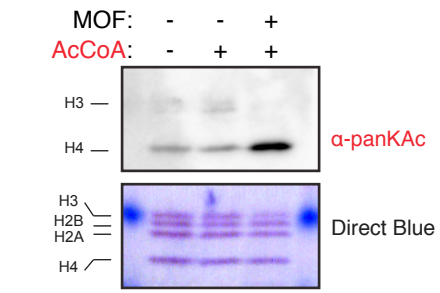






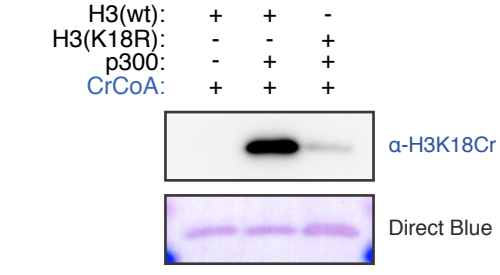


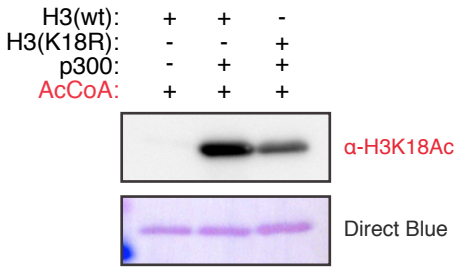




H

Histone	Lysine	Acetyl	Crotonyl
H3	9	8	-
	14	32	-
	18	70	16
	23	42	10
	27	38	5
	36	13	-
	37	7	-
	79	2	-
H4	5	13	-
	8	15	-
	12	12	-
	16	8	-
H2B	11	1	-
	12	15	-
	15	18	-
	16	28	-
	20	42	-
	23	10	-
H2A	-	-	-





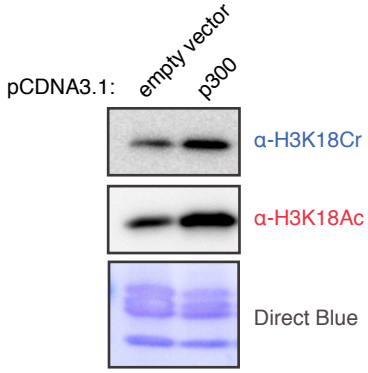
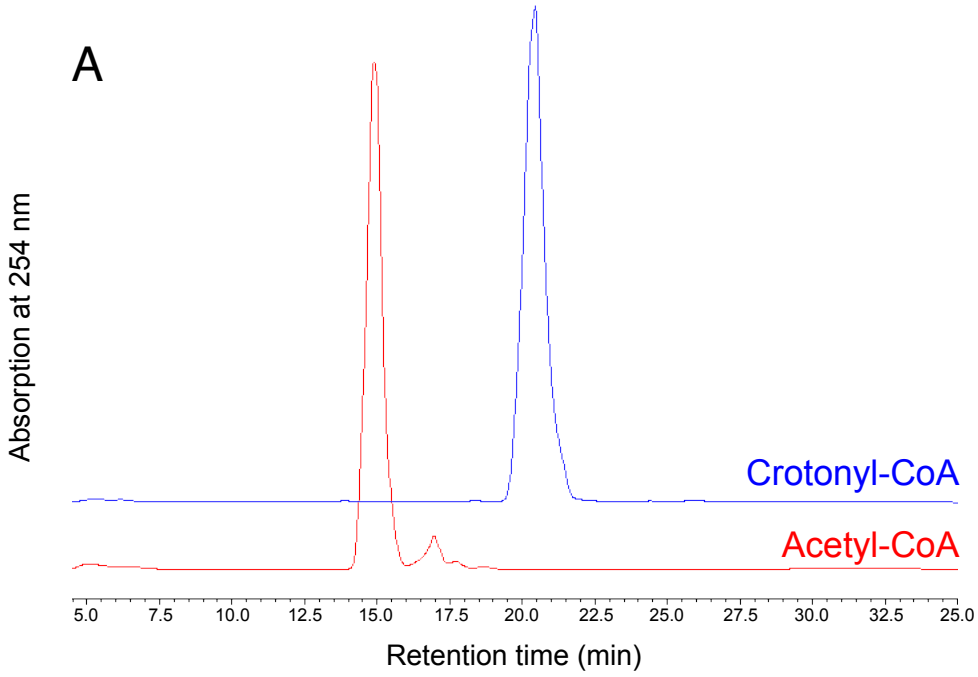


Figure S2 (Related to Figure 2)



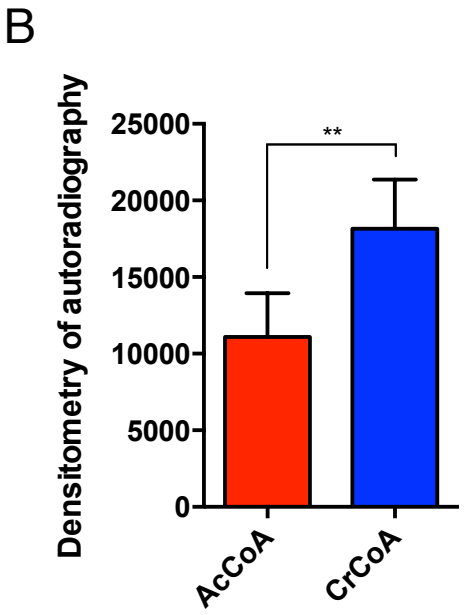


Figure S3 (related to Figure 3)

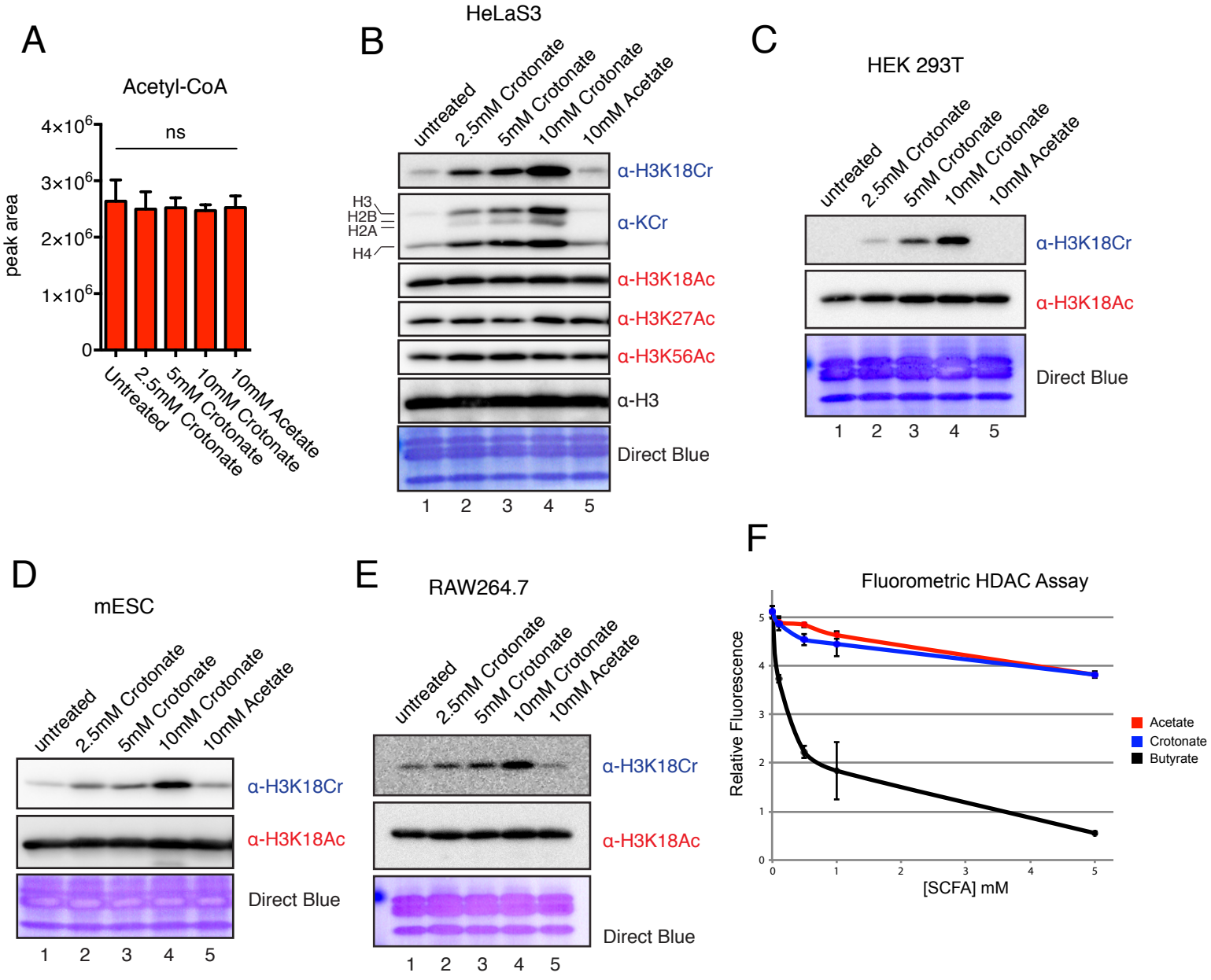


Figure S4 (related to Figure 3)

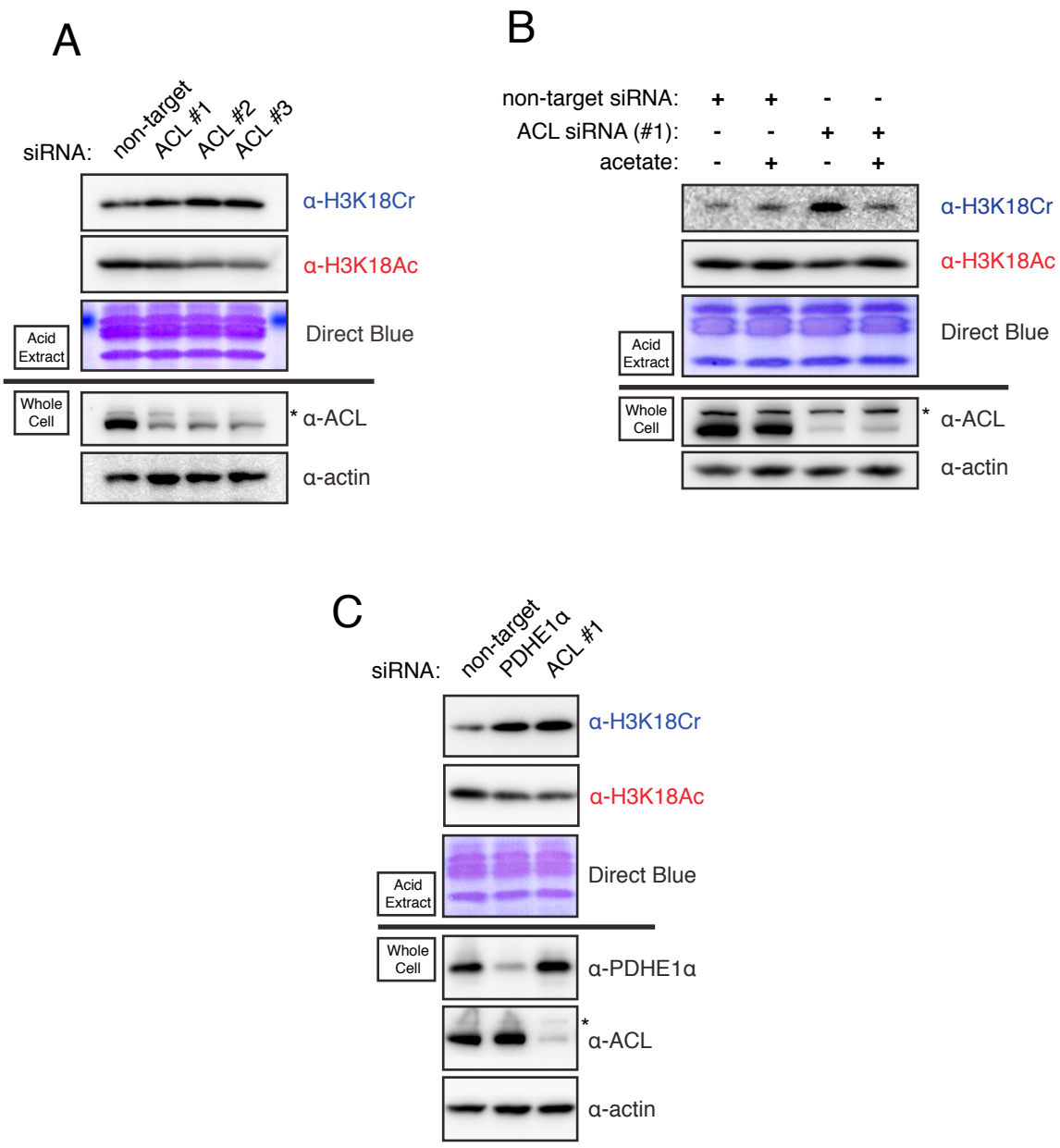


Figure S5 (related to Figure 4)

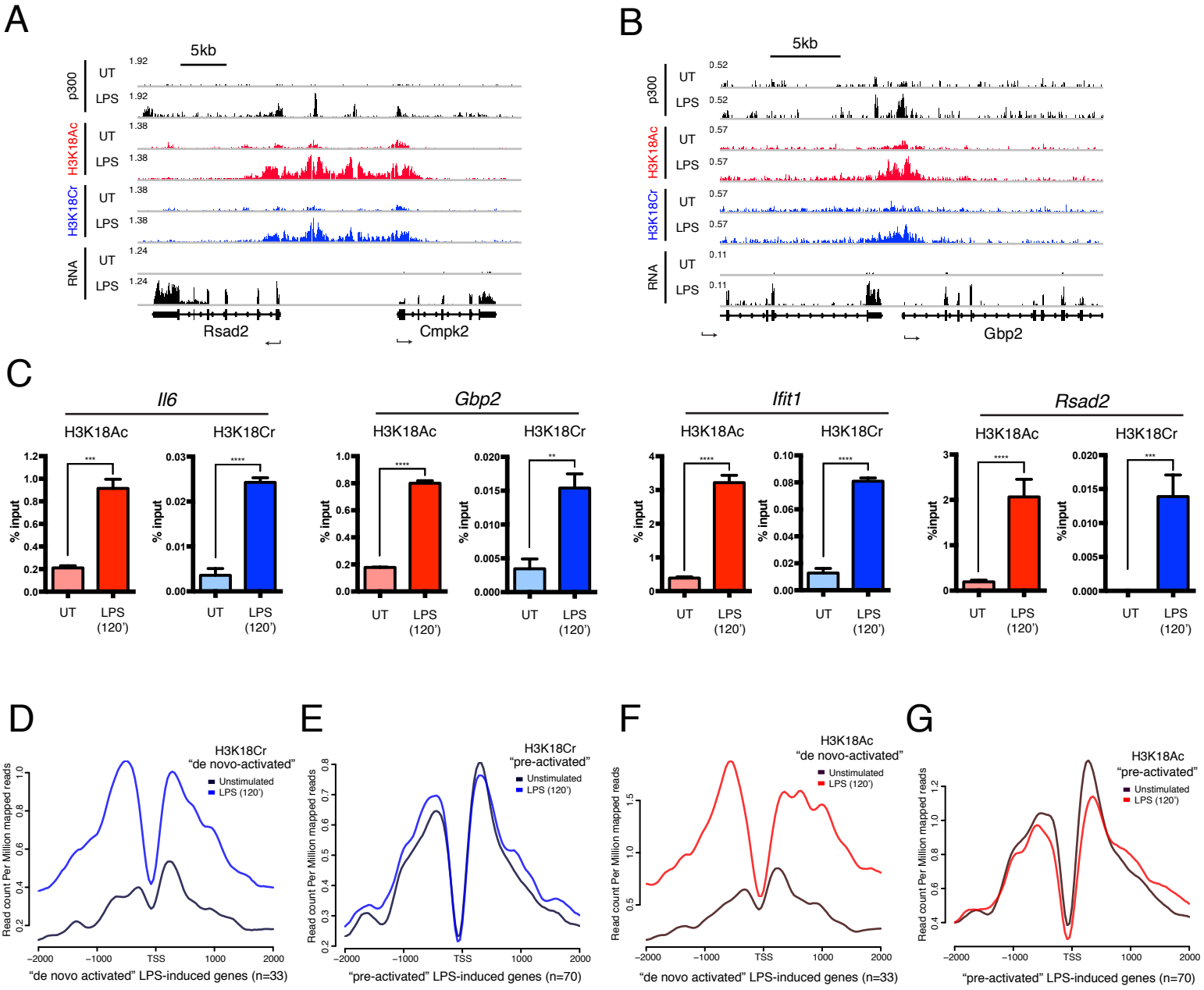
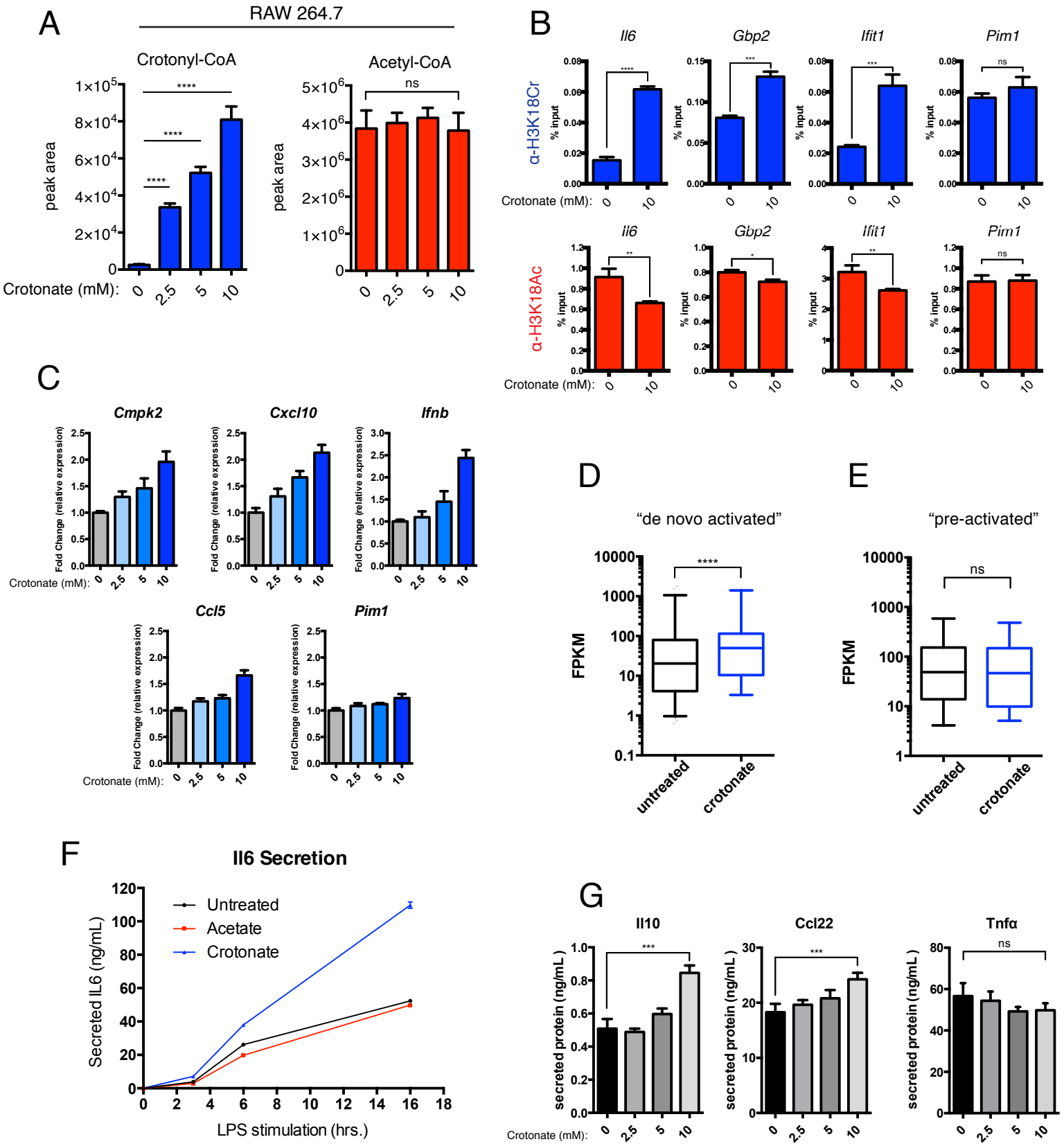


Figure S6 (related to figure 5)



SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Purification of HCT Activity Purifies p300, Related to Figure 1

(A) Chemical structures of all observed histone acylations

(B) Extended partial purification scheme

(C) Box and whisker plot representation of Intensity Based Absolute Quantification (iBAQ) values for all proteins identified in the crude nuclear extract, and in the three Mono S fractions with peak HCT activity (fractions 4, 5, and 6). Lower and upper whiskers indicate 10% and 90% whereas boxes indicate 25%, median and 75% percentiles. The similarity of the iBAQ value distributions (box plots) suggests that comparison of the crude nuclear extract and the different fractions is appropriate. Calculated iBAQ values for all observed HATs (p300, CBP, MOF, TIP60, GCN5, HBO1, HAT1) are plotted onto the box plots. All shown HATs were confidently matched with between 7 and 46 unique peptides. In the experiment the iBAQ value is used to measure relative abundance of a particular acetyl transferase in the fractions compared to the crude input. This experiment indicates that EP300 (green label) is dramatically enriched, from being among the 10% least abundant proteins in the crude input to among the 25% most abundant proteins in fractions 4 to 6. Absence of HAT1 (purple) in the fractions suggests that this acetyl transferase was lost during purification of HCT activity.

(D) Enrichments of iBAQ values for Fraction 5 over crude nuclear extract for all observed proteins are plotted as $\log_2$ values. The observed HATs are highlighted in red.

(E) Heatmap representation of the relative iBAQ value enrichment or depletion over crude nuclear extract of the seven observed HATs in Mono S fractions 4, 5, and 6.

(F-G) Recombinant MOF is used as the enzyme in HCT (F) and HAT (G) assays with recombinant octamers as substrate. Reaction products were immunoblotted with the indicated antibodies.

(H) Semi-quantitative MS/MS analysis of p300 acetylation and crotonylation of recombinant chromatin. Reaction products of p300-driven HAT or HCT assays using recombinant chromatin as substrate were subject to MS/MS analysis (see Experimental Procedures for more detail). These assays were carried out as in Figure 3B. Data is represented as spectral counts (Carvalho et al., 2008). Shade of color (red for acetyl and blue for crotonyl) represents ranking of modified residue by spectral counts. Dashes indicate that no peptides were observed with the particular modification.

(I-J) The H3K18Cr and H3K18Ac antibodies are specific for modification of H3K18. Recombinant H3 with lysine 18 mutated to arginine was purified from bacteria and used, in parallel with wild-type H3, as substrates in p300-driven HCT (I) and HAT (J) assays. Reaction products were immunoblotted with the indicated antibodies.

(K) Overexpression of p300 in HEK 293T cells increases global levels of H3K18Cr and H3K18Ac. 293T cells are transfected with p300 cDNA. 48 hours post-transfection histones were prepared and immunoblotted with the indicated antibodies.

Figure S2. p300-Catalyzed Histone Crotonylation Stimulates Transcription, Related to Figure 2

(A) Acetyl-CoA or crotonyl-CoA were injected onto a C18 HPLC column and eluted over an acetonitrile gradient as previously described (King and Reiss, 1985). Presence of CoA molecules was detected by absorbance at 254 nm. The increased hydrophobicity of crotonyl-CoA compared to acetyl-CoA causes the molecule to have an increased retention time allowing for clear separation of the two molecules.

(B) Absolute values for densitometry of autoradiographs from three independent experiments, presented as average ± standard deviation, paired t-test p value = 0.0012.

Figure S3. Addition of Crotonate Increases Global Histone Crotonylation, Related to Figure 3

(A) LC-MS analysis of cellular acetyl-CoA levels in HeLa S3 cells treated with the indicated concentration of sodium crotonate (pH 7.4) or sodium acetate (pH 7.4) for 12 hours. The data represent mean peak area ± standard deviation of four independent experiments. The p-value from an unpaired t-test of untreated to

10mM acetate treated is 0.61.

(B) Extended immunoblot analysis of Figure 2C. Histones were acid extracted form HeLaS3 cells treated with the 2.5, 5, or 10mM crotonate, 10mM acetate, or left untreated for 12 hours. The acid extracted histones were immunoblotted with the indicated antibodies.

(C-E) HEK 293T cells (C), mouse embryonic stem cells (mESC) (D), and RAW 264.7 cells (E) were treated with indicated concentration of crotonate or acetate and then processed and analyzed as in (B).

(F) Crotonate does not act as an HDAC inhibitor. Increasing concentration of either acetate, butyrate, or crotonate (0, 0.5mM, 1mM, or 5mM) were added to a Fluorometric HDAC assay kit (Active Motif) where fluorescence intensity is an indirect measure of HDAC activity. HeLaS3 nuclear extract was used as a general source of HDAC activity.

Figure S4. Reducing Acetyl-CoA Leads to an Increase in Global Histone Crotonylation, Related to Figure 3

(A) HeLa S3 cells were transfected with non-target, or one of three unique ACL siRNAs, as indicated. 72hrs post-transfection histone extracts and whole cell lysates are prepared and immunoblotted with the indicated antibodies. Immunoblots of acid extracts are shown above the black line, while immunoblots of whole cell lysates are shown bellow the black line.

(B) HeLa S3 cells were transfected with either non-target or ACL-specific siRNA

(#1) using the same protocol as in (A), except 14 hours prior to harvest 10 mM acetate was added to the media as indicated. Analysis and labeling are as in (A).

(C) HeLa S3 cells were transfected with non-target, PDHE1α, or ACL (#1) specific siRNA, as in (A-B). Analysis and labeling are as in (A-B).

Figure S5. "De Novo-Activated" Genes Show Significant De Novo Increases in H3K18Ac and H3K18Cr at Proximal Regulatory Elements, Related to Figure 4

(A-B) Genome browser representation of RNA-seq reads and ChIP-seq reads for p300, H3K18Ac, and H3K18Cr from unstimulated (UT) and 120' LPS-stimulated (LPS) macrophages at three "de novo-activated" genes (*Rsad2*, *Cmpk2*, and *Gbp2*). Normalized to total mapped reads. The y-axis maximum is given at the far left of each track. Arrow below refseq gene track indicated directionality of transcription.

(C) H3K18Ac and H3K18Cr ChIP-qPCR data for four "de novo-activated" genes either untreated (UT) or stimulated with LPS for 2 hours (LPS (120')). Data are represented as mean of % input ± standard deviation of technical replicates. Summary of p-value is as follows: ns ($p>0.05$), * ($p\leq0.05$), ** ($p\leq0.01$), *** ($p\leq0.001$), **** ($p\leq0.0001$).

(D-G) The average profile of H3K18Cr (D and E) and H3K18Ac (F and G) ChIP-seq data plotted at the TSS +/- 2000bp of either all "de novo-activated" genes (D and F) or all "pre-activated" genes (E and G) with or without LPS stimulation.

Figure S6. Crotonate Pre-Treated Cells Show Enhanced Induction of "De Novo-Activated" genes and Gene Products upon LPS stimulation, Related to Figure 5

(A) LC-MS measurements of crotonyl-CoA (left) and acetyl-CoA (right) from RAW 264.7 cells pretreated with the indicated concentration of sodium crotonate, pH 7.4, for 12 hours. The data represent mean peak area ± standard deviation of four independent experiments. Summary of unpaired t-test p-value is as follows: ns ($p>0.05$), * ($p\leq0.05$), ** ($p\leq0.01$), *** ($p\leq0.001$), **** ($p\leq0.0001$).

(B) qPCR analysis of H3K18Cr and H3K18Ac ChIP products from RAW 264.7 cells pre-treated with the indicated concentration of sodium crotonate (pH 7.4) (mM) for 6 hours prior to a 2-hour LPS stimulation. ChIP-qPCR results for three "de novo-activated" genes (*Il6*, *Gbp2*, and *Ifit1*) and one "pre-activated" gene (*Pim1*) are shown. Data are represented as mean of % input ± standard deviation of technical replicates. Summary of p-values is as in (A).

(C) RT-qPCR analysis of LPS-stimulated (120') RAW 264.7 cells pre-treated with the indicated concentration of sodium crotonate (pH 7.4) (mM). Relative expression is normalized to *Gapdh*. Data are represented as the mean fold-change in relative expression due to crotonate addition from three independent experiments ± standard deviation.

(D-E) Box and whisker plot (10^{th} to 90^{th} percentile) representation of FPKM values derived from RNAseq data of untreated or 10mM crotonate treated prior to 2hr LPS stimulation for all "de novo-activated" genes (D) or all "pre-activated"

genes (E). P-values are from ratio paired t-tests, the summary of p-values are as in (A).

(F) RAW 264.7 cells were pretreated with either 10mM Acetate or 10mM Crotonate, or left untreated for 6 hours and then LPS stimulated. Supernatants were collected at 0, 3hr, 6hr, or 16hr after LPS stimulation and the concentration of Il6 was measure by standard ELISA. Data are presented as mean of technical replicates ± standard deviation.

(G) Supernatants were collected from RAW 264.7 cells pre-treated with the indicated concentration of crotonate and LPS stimulated for 16hrs and subject to a bead-based immunoassay to measure the concentration of various cytokine and chemokines. Data for two “de novo-activated” chemo/cytokines (IL10 and CCL22) and one “pre-activated” chemokine (TNFα) are represented here as the mean of two independent experiments ± standard deviation. Unpaired t-test p-value summaries are as in (A).

Table S1. MaxQuant Output from MS/MS Analysis of Crude Extract and Peak Mono S Fractions, Related to Figure 1

To identify proteins enriched by the purification of the HCT activity, crude nuclear extract and Mono S fractions 3, 4, and 5 were subject to MS/MS analysis. MaxQuant was used to analyze data and the ‘proteinGroups.txt’ output is presented here. See Extended Experimental Procedures for technical details.

EXTENDED EXPERIMENTAL PROCEDURES

HeLa S3 Nuclear Extract Preparation

Eight liters of HeLa S3 suspension culture (~1x10^10 cells) were harvested by centrifugation and then washed in cold PBS. The pellet was resuspended in hypotonic lysis buffer (20 mM HEPES pH 7.9, 10 mM KCl, 5 mM $MgCl_2$, 0.5 mM EGTA, 0.1 mM EDTA, 5 mM βME, 1 mM sodium metabisulfite, 1 mM benzamidine, 0.8 mM PMSF) and dounced ten times with pestle A and ten times with pestle B or until >90% of cells were determined lysed by trypan blue staining. Lysed cells were centrifuged at 3000 rpm to pellet intact nuclei. Nuclei were resuspended in a nuclear resuspension buffer (20 mM HEPES pH 7.9, 110 mM KCl, 2 mM $MgCl_2$, 0.1 mM EDTA, 5 mM BME, 1x complete protease inhibitor (Roche), 0.4 mM PMSF) and dounced ten times with pestle A to homogenize the suspension. Solid ammonium sulfate was added to a final concentration of 400 mM, samples were immediately mixed by inversion, and left rotating at 4°C for 30 minutes. The samples were then clarified by centrifugation at 35,000 rpm for 1 hour. The supernatant is the soluble nuclear extract. The soluble nuclear extract was then subject to ammonium sulfate precipitation/salting out to 60% saturation. The precipitate was collected and gently resuspended in buffer D (20mM HEPES pH 7.9, 100 mM KCl, 1 mM EDTA, 10% glycerol, 1 mM DTT, 1 mM PMSF) and dialyzed twice against 2 L of Buffer D for 2 hours. The dialyzed extract is the crude nuclear extract that was subject to further fractionation.

Recombinant Protein Purification

Recombinant p300, p53, and MOF were expressed in Sf9 cells by baculovirus infection and purified by FLAG immuno-precipitation (IP) of nuclear extracts. Recombinant p300 and p53 were purified as described previously (Tang et al., 2013). MOF cDNA (MHS6278-202808687) was cloned into pFastbac1 with a N-terminal FLAG tag. Baculovirus was generated by the Bac-to-bac system per manufacturer's instructions (Life). Amplified baculovirus was used to transduce sf9 cells and MOF protein was purified by FLAG IP. The catalytic domains of GCN5 (residues 505-670) and TIP60 (residues 181-462) were cloned from HeLaS3 cDNA libraries into pGEX6p1 to generate GST-fusion proteins. Rosseta2(DE3)pLysS were transformed, induced by IPTG, and soluble protein was purified by standard glutathione-sepharose chromatography.

In Vitro Assays

HAT/HCT assays were incubated at 30°C for 1 hour in Buffer A (50 mM Tris pH 8.0, 10% glycerol, 1 mM DTT, 0.2 mM PMSF). When extract was used as an enzyme source 10mM Sodium Butyrate was included in Buffer A. 2-100 μM of either crotonyl-CoA or acetyl-CoA (Sigma 28007 and A2181) was used as the high-energy acyl donor. Unless otherwise noted, 100 μM was used in initial purifications, 2 μM in subsequent HAT and HCT in vitro assays, and 50 uM in the transcription assays. The oligonucleosome substrate was sucrose-gradient purified from micrococcal nuclease-digested HeLaS3 nuclei, as previously

described (Fang et al., 2003). Recombinant chromatin used in p300 reactions and transcription assays was prepared as described (Tang et al., 2013). Recombinant histone proteins were purified and assembled into octamers as previously described (Ruthenburg et al., 2011). Under reaction conditions the "octamers" are most likely present as H3-H4 tetramers and H2A-H2B dimers, while oligonucleosome and recombinant chromatin substrate will maintain their structure. Fluorescence-based HDAC assays were performed using an HDAC Assay Kit (Active Motif 56200) according to manufacturer's instruction. p53-dependent in vitro transcription assays were performed as previously described (Tang et al., 2013). Densitometry of autoradiographs was performed with ImageJ.

ChIP

RAW 264.7 cells were grown in full media, pretreated with the indicated concentration of crotonate for six hours, and then stimulated with 100 ng/mL of LPS for two hours. Chromatin from ~$5x10^7$ cells was used per immuno-precipitation. Media was aspirated and cells washed with PBS followed by addition of 1% formaldehyde (in PBS). Cells were cross-linked directly on the plate for 10 minutes with gentle shaking. Glycine was added to a final concentration of 125 mM to quench the crosslinking and allowed to incubate for 5 minutes. Cells were scraped off the plates, pelleted, and washed in PBS plus 1x Complete protease inhibitors (Roche), 10 mM Butyrate, and 10 mM nicotinamide. Pellets were resuspended in 10mL LB1 (50 mM HEPES, 140 mM NaCl, 1 mM

EDTA, 10% glycerol, 0.5% NP40, 1% TritonX-100, 1x Complete protease inhibitor (Roche), 10 mM Butyrate, 10 mM nicotinamide) and incubated rotating at 4°C for 20 minutes. Samples were centrifuged and pellets were resuspended in 10 mL LB2 (10 mM Tris pH 8.0, 200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 1x Complete protease inhibitors (Roche), 10 mM butyrate, 10 mM nicotinamide). Samples were incubated rotating at 4°C for 10 minutes and then centrifuged. Pellets were resuspended in 1.2 mL LB3 (10 mM Tris pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% sodium-deoxycholate, 0.5% sodium lauroyl sarcosinate, 1% TritonX-100, 1x Complete protease inhibitors (Roche), 10 mM butyrate, 10 mM nicotinamide) and passed through a 27-gauge needle until suspension was homogenized. The samples were then sonicated in a Bioruptor cooled water bath sonicator (Diagenode) for 60 cycles of 30' on 30' off on high intensity. Samples were clarified by centrifugation at 16,000 g for 15 minutes. The supernatant is the soluble chromatin extract. 5% of input volume was set aside. 7.5 µg of H3K18Cr or H3K18Ac antibody were conjugated to 75 µL protein A Dynabeads (Novex) per immuno-precipitation. After unbound antibody was washed away, the beads were added to the clarified soluble chromatin extract and incubated rotating overnight at 4°C. A magnetic stand was used to separate beads from the extract and beads were washed under the following conditions: three times with 1 mL wash buffer 1 (50 mM HEPES, 100 mM LiCl, 1 mM EDTA, 1% NP-40, 0.7% sodium deoxycholate, 10 mM butyrate, 10 mM nicotinamide), three times with 1mL wash buffer 2 (20mM Tris-HCl pH 8.0, 350mM NaCl, 1% TritonX-100, 0.1% SDS, 2mM EDTA, 10 mM butyrate, 10 mM nicotinamide), and

once with 1 mL buffer TE plus 50 mM NaCl. The washed beads were resuspended in 210 µL elution buffer (50 mM Tris pH 8.0, 10 mM EDTA, 1% sodium dodecyl sulfate) and incubated at 65°C for 30 minutes while shaking to prevent beads from settling. The eluate was transferred to a new tube, 5% inputs were brought up to equal volume with elution buffer, and both were incubated at 65°C overnight to reverse crosslinks. The samples were then treated with RNase and Proteinase K. DNA was recovered using the Qiagen PCR purification kit according to manufacturer's instruction. A 1/100 dilution of each sample was used for qPCR analysis as described in experimental procedures.

ChIP-qPCR

Cross-linking ChIP was performed with anti-H3K18Ac (Abcam 1191) and anti-H3K18Cr (PTM-biolabs 517). ChIP products and inputs were analyzed by qPCR using Power SYBR Green (Life) and StepOnePlus Real-Time PCR system (Applied Biosystems). The sequences of ChIP-qPCR primer pairs with forward followed by reverse are: *Il6* (AATGTGGGATTTTCCCATGA/ GCTCCAGAGCAGAATGAGCTA), *Gbp2* (GCCCAGGGCTAGGTGACA/ TGTGAAGTCTTCTTTCCCAGAGTTT), *Ifit1* (GCCATCCTAAGACCCCCTAGTG/ TCTGCAGTTCCTCCTTGGAAGT), *Pim1* (CTGGCCTCGGTGTGCAA/ GGCGAAGGCTGTGCAAGA), *Rsad2* (CAATCCCAACTCCTTTCCCAACA/ TCTGACCTCCATAACCAAATGAACT), *Ccl3* (ATCTCCAGCTCGAGCAATGG/ AGTCACTTTGCGGCTGATGA), *Ccl5* (TGAGCCTTTGAGGAGGTTGG/ CACTGCAAGTCACGGCCATA). Melt-curve analysis along a dilution series was

carried out to ensure unique product amplification for all primer pairs. All statistical analysis was performed using PRISM v6.0 (Graph Pad).

RT-qPCR

Total RNA was extracted using the RNeasy Kit (Qiagen) and Dnase treated on column according to manufacturer's instructions (Qiagen). cDNA was prepared using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to manufacturer's instructions. cDNA fragments were analyzed by qPCR using Power SYBR Green (Life) and StepOnePlus Real-Time PCR system (Applied Biosystems). A standard curve for each primer pair was used to ensure linear dilution and to quantitate relative cDNA abundance. The sequences of RT-qPCR primer pairs with forward followed by reverse are: *Il6* (GTTCTCTGGGAAATCGTGGA/ TTTCTGCAAGTGCATCATCG), *Gbp2* (CTCTACCGCACAGGCAAATC/ GATGCCCTTGGTGTGAGACT), *Ifit1* (AGTGAGGTCAACCGGGAATCT/ TCTAGGTGCTTTATGTAGGCCA), *Pim1* (TCAAGGACACAGTCTACACGG/ AGCGATGGTAGCGAATCC), *Rsad2* (AACCCCCGTGAGTGTCAACTA/ AACCAGCCTGTTTGAGCAGAA), *Ccl3* (TACAGCCGGAAGATTCCACG/ TCAGGAAAATGACACCTGGCT), *CCL5* (GTGCCCACGTCAAGGAGTAT/ CCCACTTCTTCTCTGGGTTG), *Cmpk2* (GACCTAGTTGACCAGTGCCC/ AGTGTGGTCTTACCAGTGGC), *Cxcl10* (ATGACGGGCCAGTGAGAATG/ TCGTGGCAATGATCTCAACAC), *Ifnb* (TGGGAGATGTCCTCAACTGC/ CCAGGCGTAGCTGTTGTACT) and *Gapdh* (TGGTGAAGGTCGGTGTGAAC/ CCATGTAGTTGAGGTCAATGAAGG). Melt-

curve analysis along a dilution series was carried out to ensure unique product amplification for all primer pairs. All statistical analysis was performed using PRISM v6.0 (Graph Pad).

RNA-seq

RNA-seq libraries were prepared using the TruSeq RNA Sample Prep Kit (Illumina) as per manufacturer's instruction. The procedure includes purification of poly-adenylated RNAs. Libraries were sequenced with 50bp single read sequencing on the HiSeq2500 (Illumina). Sequencing data was processed and analyzed as described in (Trapnell et al., 2012)

ChIP-seq

ChIP-seq libraries were prepared using the TruSeq ChIP Sample Prep Kit (Illumina) as per manufacturer's instruction. Libraries were sequenced with 50bp single read sequencing on the HiSeq2500 (Illumina). 50bp reads were aligned using bowtie (v1.0.1) to the mouse mm9 reference assembly reporting only unique reads in the best stratum, with up to 2 mismatches (bowtie -p 8 -S -q -v 2 -m 1 -k 1 --best –strata). Samtools (v0.1.19) was then used to convert files to bam format, sort, and remove PCR duplicates (Li et al., 2009). For genome browser representation, IGV files were generated by IGVtools (v2.3.7) and data was visualized using IGV (v2.1.28) (Thorvaldsdottir et al., 2013). Ngs.plot (v2.08) was used to generate average profiles of ChIPseq reads (Shen et al., 2014). MACS (v1.4.2) was used to call peaks (Zhang et al., 2008). BEDOPs (v2.4.1),

bedmap --basses function, was used for read counting within specified genomic regions (Neph et al., 2012).

Immuno-detection of Secreted Inflammatory Mediators

Supernatants from RAW 264.7 cells under indicated conditions and for indicated length of LPS stimulation were assayed by two distinct immuno-assays. A standard immobilized ELISA for mouse Il6 (Life KMC0061) was used as per manufacturer's instruction. And a bead-based assay that uses the principles of sandwich ELISA to quantify soluble analytes using a flow cytometer (Biolegend: Legendplex assay) was used to quantify the concentration of a number of secreted chemokines and cytokines, as per manufacturer's instruction.

Mass Spectrometry Analysis of p300 Reaction Products

Recombinant chromatin was acetylated or crotonylated by p300 in the presence of p53 and reaction products were resolved on an 8% Tris-Glycine SDS-PAGE gel. The gel bands of core histone proteins were excised and subjected to in-gel tryptic digestion. The tryptic digests were desalted with Ziptip C18 and loaded onto a homemade capillary column (10 cm length with 75 um inner diameter) packed with Jupiter C12 resin (4 µm particle size, 90 Å pore size, Phenomenex Inc.) connected to a NanoLC-1D plus HPLC system (Eksigent Technologies LLC, Dublin, CA). Peptides were eluted with a gradient of 5% to 90% HPLC buffer B (0.1% formic acid in acetonitrile, v/v) in buffer A (0.1% formic acid in water, v/v) at

a flow rate of 300 nL/min over 76 min. The eluted peptides were ionized and introduced into a LTQ-Orbitrap Velos mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA) using a nanospray source. Full MS scans were acquired in the Orbitrap mass analyzer over the range m/z 300-1800 with a mass resolution of 60000 at m/z 400. Twenty of the most intense ions were isolated for MS/MS analysis. Database searching was performed against the NCBI protein sequence database, using Mascot. The database searching parameters included: lysine acetylation, protein N-terminal acetylation, lysine crotonylation, lysine trimethylation, lysine/arginine di-methylation, lysine/arginine mono-methylation, and methionine oxidation as variable modifications. All the identified peptides were manually verified.

Mass Spectrometry Analysis of Mono S Fractions

50 µL of each fraction was precipitated overnight using 500 µL ice cold acetone. Same process was followed for the 'input' sample with the exception that sample volume was 100 µL and 1000 µL ice cold acetone was used for precipitation. Acetone was carefully removed, first by suction, then by evaporation. Precipitates was dissolved in 30 µL 8M Urea/0.1M ammonium bicarbonate/10mM Dithiothreitol and vortexed at room temperature for 45 minutes. Reduced proteins was alkylated in the dark by adding 7 µL 100mM iodoacetaminde to each vial. Samples was then diluted 2-fold with 0.1M ammonium bicarbonate and 1ug Endopeptidase Lys-C (Wako Chemicals USA, Inc, Richmond, VA) in 10 µL in 0.1M ammonium bicarbonate was added to each vial. Digestion was allowed to

proceed for 6h. Samples were further diluted by adding 80 µL 0.1M ammonium bicarbonate prior to the addition of 1ug trypsin (Promega, Madison, WI, USA) dissolved in 20 µL 0.1M ammonium bicarbonate. Trypsination of the samples was allowed to proceed overnight, and the digestion was halted by adding 2 µL trifluroacetic acid. Half of each sample was desalted using in-house SPE columns made with Empore C_{18} (Rappsilber et al., 2003). Generated peptides were measured by nano LC-MS/MS using a Q-Exactive mass spectrometer (Thermo, Bremen, Germany) coupled to a Dionex NCP3200RS HPLC setups (Thermo, Sunnyvale, CA, USA). Samples were desalted and concentrated on a trap column prior to separation on a packed-in-emitter C_{18} column (75 µm by 12 cm, 3 µm particles - Nikkyo Technos Co., Ltd. Japan). The analytical gradient was generated at 300 nL/min increasing from 10% Buffer B (0.1% formic acid in acetonitrile) / 90% Buffer A (0.1% formic acid) to 45% Buffer B / 55% Buffer A in 137 minutes, followed by a wash step with 90% Buffer B / 10% Buffer A for 16 minutes and conditioning for 10 minutes with 1% Buffer B / 99% Buffer A Buffer B. Data Dependent Acquisition experiment: MS survey scans was scanned from m/z 300 to m/z 1400 at resolution of 70,000@200 Th (AGC: 5e5 and maximum IT: 100 ms). Up to the 20 most abundant ions were subjected to MS/MS and measured at a resolution of 17,500 (AGC: 5e5 and maximum IT: 60 ms) with m/z 100 as lowest mass. Precursor ions were isolated at 2.0 Th. Lock mass of m/z 371.10123 was used for all measurements. All samples were analyzed in technical duplicates. Data were searched and quantified using MaxQuant 1.5.30 (Cox et al., 2014). Technical replicates were merged for the analysis. The Uniprot

Complete Proteome human protein database (July 2014) was queried. Match between runs was utilized. Combining all samples approximately 3,700 proteins were matched at 1% protein and peptide False Discovery Rate. For the comparison of the data set intensity Based Absolute Quantitation (iBAQ) (Schwanhäusser et al., 2012) was used. MaxQuant 'proteinGroups.txt' output is available in Table S1.

Mass Spectrometry Analysis of Acetyl-CoA and Crotonyl-CoA

HeLa S3 and RAW 264.7 cells were maintained in DMEM medium supplemented with 10% FBS, 2 mM of L-glutamine, 100 units/mL penicillin and 100 ug/mL streptomycin at 37°C under an atmosphere of 5% of CO_2. The acyl-CoA extraction procedure was based upon the method previously described by Basu and Blair, 2011, with minor modifications. Cultured cells were washed once with cold deionized water and metabolites extracted by the addition of 1 mL of ice cold 10% trichloroacetic acid (TCA). Following 15 seconds incubation on ice, cells were collected by scrapping and transferred to a 1.5 mL Eppendorf tube. Cell extracts were sonicated for 3 x 30 seconds (Bioruptor, Diagenode) and then centrifuged at 14,000 rpm for 5 minutes at 4°C to remove protein. Acyl-CoAs were further purified using a HLB 96-well plate solid phase extraction plate (30 mg) and vacuum manifold (Waters Corporation). SPE wells were conditioned with 1 mL of methanol, and equilibrated with 1 mL of water. Supernatants were applied and then SPE wells were washed with 1 mL of water; acyl-CoAs were eluted using three successive applications of 0.5 mL of methanol containing 25

mM ammonium acetate. Eluted acyl-CoAs were dried for 5 hours in a bench top solvent evaporator (Genevac EZ-2 Elite). Dried samples were stored at -80°C and re-suspended in 100 µL of 5% 5-sulfosalicylic acid (SSA) immediately prior to analysis by liquid chromatography/mass spectrometry (LC/MS).

Acyl-CoA were separated using a Phenomenex Luna C18 column (2.0 mm x 50 mm, 5 µm) using 10 mM ammonium acetate in water, pH 8 as the aqueous mobile phase and 90% acetonitrile, 10% water with 10 mM ammonium acetate, pH 8 as the organic phase. The injection volume was 15 µl (Accela Open Autosampler, Thermo Fisher Scientific) and a constant column temperature was maintained at 30°C using a thermostatically controlled column oven (MayLab). LC-20A pumps (Shimadzu) were used for the primary LC gradient: 0 min, 3% B; 1 min, 3% B; 6 min, 40% B; 7 min, 40% B; with an additional 6 min allowed for column re-equilibration between injections. A constant flow rate of 0.2 mL/min was maintained, with an additional 0.1 mL/min of 90% acetone, 10% dimethyl sulfoxide added post-column by means of an Accela 1250 pump (Thermo Fisher Scientific) and PEEK tee, to increase the sensitivity of acyl-CoAs detection.

Acyl-CoAs were detected using a TSQ Vantage triple quadrupole mass spectrometer (Thermo Fisher Scientific) equipped with H-ESI II probe, operating in positive ionization mode. MS parameters were: spray voltage, 3000 V; sheath gas pressure, 35 psi; auxiliary gas pressure, 30 psi; capillary temperature, 270°C; and vaporizer temperature, 250°C. Data acquisition was performed in single reaction monitoring (SRM) mode using S-lens of 200 V, collision energy of

26 eV, 50 millisecond scan time and compound specific parameters: Acetyl-CoA 810.1 → 303.1 m/z, Crotonyl-CoA 836.1 → 329.1 m/z. Data was acquired and analyzed using TraceFinder 3.1 software (Thermo Fisher Scientific).

SUPPLEMENTAL REFERENCES

Basu, S.S., and Blair, I.A. (2011). SILEC: a protocol for generating and using isotopically labeled coenzyme A mass spectrometry standards. Nat Protoc *7*, 1–12.

Carvalho, P.C., Hewel, J., Barbosa, V.C., and Yates, J.R. (2008). Identifying differences in protein expression levels by spectral counting and feature selection. Genet. Mol. Res. *7*, 342–356.

Cox, J., Hein, M.Y., Luber, C.A., Paron, I., Nagaraj, N., and Mann, M. (2014). Accurate proteome-wide label-free quantification by delayed normalization and maximal peptide ratio extraction, termed MaxLFQ. Molecular & Cellular Proteomics *13*, 2513–2526.

Fang, J., Wang, H., and Zhang, Y. (2003). Purification of histone methyltransferases from HeLa cells. Meth. Enzymol. *377*, 213–226.

King, M.T., and Reiss, P.D. (1985). Separation and measurement of short-chain coenzyme-A compounds in rat liver by reversed-phase high-performance liquid chromatography. Analytical Biochemistry *146*, 173–179.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., 1000 Genome Project Data Processing Subgroup (2009). The Sequence Alignment/Map format and SAMtools. Bioinformatics *25*, 2078–2079.

Neph, S., Kuehn, M.S., Reynolds, A.P., Haugen, E., Thurman, R.E., Johnson, A.K., Rynes, E., Maurano, M.T., Vierstra, J., Thomas, S., et al. (2012). BEDOPS: high-performance genomic feature operations. Bioinformatics *28*, 1919–1920.

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.

Ruthenburg, A.J., Li, H., Milne, T.A., Dewell, S., McGinty, R.K., Yuen, M., Ueberheide, B., Dou, Y., Muir, T.W., Patel, D.J., et al. (2011). Recognition of a Mononucleosomal Histone Modification Pattern by BPTF via Multivalent Interactions. Cell *145*, 692–706.

Schwanhäusser, B., Busse, D., Li, N., Dittmar, G., Schuchhardt, J., Wolf, J., Chen, W., and Selbach, M. (2012). Global quantification of mammalian gene expression control. Nature *473*, 337–342.

Shen, L., Shao, N., Liu, X., and Nestler, E. (2014). ngs.plot: Quick mining and visualization of next-generation sequencing data by integrating genomic databases. BMC Genomics *15*, 284.

Tang, Z., Chen, W.-Y., Shimada, M., Nguyen, U.T.T., Kim, J., Sun, X.-J., Sengoku, T., McGinty, R.K., Fernandez, J.P., Muir, T.W., et al. (2013). SET1 and p300 Act Synergistically, through Coupled Histone Modifications, in Transcriptional Activation by p53. Cell *154*, 297–310.

Thorvaldsdottir, H., Robinson, J.T., and Mesirov, J.P. (2013). Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. Briefings in Bioinformatics *14*, 178–192.

Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D.R., Pimentel, H., Salzberg, S.L., Rinn, J.L., and Pachter, L. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat Protoc *7*, 562–578.

Zhang, Y., Liu, T., Meyer, C.A., Eeckhoute, J., Johnson, D.S., Bernstein, B.E., Nussbaum, C., Myers, R.M., Brown, M., Li, W., et al. (2008). Model-based Analysis of ChIP-Seq (MACS). Genome Biol *9*, R137.