Histone posttranslational modifications (PTMs) regulate chromatin dynamics, DNA accessibility, and transcription to expand the genetic code. Many of these PTMs are produced through cellular metabolism to offer both feedback and feedforward regulation. Herein we describe the existence of Lys and Arg modifications on histones by a glycolytic by-product, methylglyoxal (MGO). Our data demonstrate that addition of histones by MGO is an abundant modification, present at the same order of magnitude as Arg methylation. These modifications were detected on all four core histones at critical residues involved in both nucleosome stability and reader domain binding. In addition, MGO treatment of cells lacking the major detoxifying enzyme, glyoxalase 1, results in marked disruption of H2B acetylation and ubiquitylation without affecting H2A, H3, and H4 modifications. Using RNA sequencing, we show that MGO is capable of altering gene transcription, most notably in cells lacking GLO1. Finally, we show that the deglycase DJ-1 protects histones from addition by MGO. Collectively, our findings demonstrate the existence of a previously undetected histone modification derived from glycolysis, which may have far-reaching implications for the control of gene expression and protein transcription linked to metabolism.

Histone posttranslational modifications (PTMs) regulate chromatin structure, DNA accessibility, and transcription to expand the genetic code. The side chains of Lys and Arg are subject to numerous PTMs, including acetylation (Lys), methylation (Lys and Arg), citrullination (Arg), ubiquitylation (Lys), and various chain-length acylations (Lys) (2). Many of these PTMs are directly linked to cellular metabolism, mediating feedback mechanisms that maintain homeostasis (3, 4). As a result, histone PTMs are tightly controlled through their enzymatic addition (“writers”) and removal (“erasers”) (3). There is growing interest in electrophilic modifications to histones (5). N-formylation of Lys residues is a nonenzymatic modification stemming from endogenous formaldehyde (6). This PTM is not only abundant (0.04–0.1% of histone Lys), but also resides at numerous epigenetic “hotspots,” including H3K18, H3K23, H4K12, and H4K31 (7). Furthermore, we recently reported the modification of histones by the lipid electrophile 4-oxo-2-nonenal (8). These PTMs are elevated during bouts of inflammation, reside at critical Lys residues (e.g., H3K23, H3K27), and are substrates for the Lys deacylase sirtuin 2 (9, 10).

Cellular metabolism produces primary and secondary intermediates capable of reacting with Lys and Arg residues (11, 12). One such metabolite, methylglyoxal (MGO), is generated as a by-product of glycolysis, existing in micromolar quantities in eukaryotic cells (13). This α-oxoaldehyde generates stable protein adducts with the amine-functional groups of Arg and Lys side chains (Fig. 1). To prevent accumulation of MGO, glyoxalase 1 (GLO1) isomerizes the labile, spontaneous hemithioacetal product of the reaction of MGO with GSH to generate lactoylglutathione (14). In a recent study, Qi et al. (15) found significantly elevated MGO levels in patients with diabetic nephropathy, along with an inverse correlation between GLO1 expression and the severity of diabetic nephropathy. Indeed, shRNA knockdown of GLO1 in vivo is known to result in increased MGO-derived protein adducts and inflammatory signaling (16, 17). In addition to diabetes, MGO concentrations are also elevated in cancer, cardiovascular disease, and renal failure (18–20). Although there is a clear link between MGO and MGO-derived PTMs in disease, protein targets for MGO are seldom investigated.

Due to the Arg- and Lys-rich nature of histones, we hypothesized that these proteins would be susceptible to addition by MGO. Here we describe the first report of histone modification by a glycolytic by-product in intact chromatin. These PTMs exist basally in cells and in mouse tissues and are elevated during hyperglycemia. Furthermore, MGO-derived PTMs are present at levels comparable to those of other canonical Lys and Arg modifications. Elevations in MGO PTMs are known to decrease H2B acetylation and ubiquitylation, likely a result of the high degree of modification present on H2B. Our findings identify a quantitatively significant histone modification linked to metabolic flux through glycolysis.

Significance

Chromatin comprises the approximately 3 billion bases in the human genome and histone proteins. Histone posttranslational modifications (PTMs) regulate chromatin dynamics and protein transcription to expand the genetic code. Herein we describe the existence of Lys and Arg modifications on histones derived from a glycolytic by-product, methylglyoxal (MGO). These PTMs are abundant modifications, present at similar levels as those of modifications known to modulate chromatin function and leading to altered gene transcription. Using CRISPR-Cas9, we show that the deglycase DJ-1 protects histones from addition by MGO. These findings demonstrate the existence of a previously undetected histone modification and provide a link between cellular metabolism and the histone code.

Author contributions: J.J.G., W.N.B., and L.J.M. designed research; J.J.G., J.A.W., P.J.K., M.M.M., and K.L.R. performed research; M.D.S., O.R.W., T.W., and D.A.S. contributed new reagents/analytic tools; J.J.G., J.A.W., M.M.M., W.N.B., D.A.S., and L.J.M. analyzed data; and J.J.G. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

This open access article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-NDD).

1To whom correspondence should be addressed. Email: larry.marnett@vanderbilt.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1802901115/-/DCSupplemental.

Published online August 27, 2018.
Results

MGO Is an Abundant Cellular Metabolite That Modifies Chromatin. To explore MGO-derived PTMs, we first established a chromatographic method for the detection of methylglyoxal hydromidazole (MG-H) 1, MG-H2, MG-H3, and \(N^\alpha\)-carboxyethyl arginine (CEA) using QuARKMod, a recently described MS-based method for quantifying Arg and Lys PTMs (21). Using stable isotope-labeled standards for each MGO-derived PTM (Supplementary Appendix, Fig. S1), we measured adducts on purified histone H4 treated with 100 \(\mu\)M MGO for 24 h. As shown in Supplementary Appendix, Figs. S1 and S2, we achieved chromatographic separation of each MG-H isomer, although only MG-H1 was detected in treated samples. The bottom trace in Supplementary Appendix, Fig. S2 shows quantifiable levels of MG-H1 and an absence of MG-H2 in chromatin fractions isolated from HEK293 cells. Although MG-H2 is stable, it has not been observed in physiological systems, and our results support this finding (22). MG-H3 was also not observed due to its hydrolysis to yield CEA (Supplementary Appendix, Fig. S1).

We next quantified cellular MGO in seven distinct cell lines cultured in low-glucose (5 mM) medium for 24 h. As shown in Fig. 2A, MGO is present basally at varying levels. Although no quantifiable MGO was detected in NIH 3T3, this is likely due to the limits of detection with this method. We next measured PTMs in chromatin fractions isolated from each cell line. As shown in Fig. 2B–D, MG-H1 and CEA are present basally and at levels comparable to those of asymmetric dimethyl Arg (ADMA), which plays a critical role in transcriptional regulation (23).

GLO1 Knockout Results in Elevated Levels of MGO and MGO-Derived Protein Adducts. GLO1 is reportedly the major enzyme responsible for the detoxification of MGO, and its expression is significantly decreased in diabetic patients (15). We hypothesized that loss of GLO1 enzymatic activity would increase the modification by MGO, generating \(N^\alpha\)-(carboxyethyl)lysine (CEL). The levels of this PTM were found to be at least an order of magnitude lower than those of Arg adducts and often below the limit of detection.

CEA Adducts Are Significantly Elevated with Increased Glycolytic Flux. The bulk of MGO is generated through glycolysis (13). We sought to quantify alterations in chromatin PTMs as a result of increased glycolytic flux using stable-isotope labeling of amino acids in cell culture (SILAC). Isotopically labeled light cells (natural abundance isotope amino acids) were cultured in the presence of 5 mM glucose, whereas isotopically labeled heavy cells (\(^{13}\)C\(^6\)15N\(_2\) Lys, \(^{13}\)C\(^6\)15N\(_4\) Arg, and \(^{13}\)C\(^6\)15N Leu) were cultured in the presence of 25 mM glucose for 24 h (Fig. 3A). As shown in Fig. 3B, cells cultured in the presence of 25 mM glucose exhibited an approximate fivefold increase in cellular MGO.

We next measured the heavy:light ratios (25 mM glucose:5 mM glucose) of both canonical (Fig. 3C) and MGO-derived (Fig. 3D) PTMs on chromatin fractions. A significant elevation in acetylated Lys (acLys) was observed following hyperglycemia, consistent with previous reports (24). In addition, a significant increase in CEA was observed following hyperglycemia; however, no such increase was observed with MG-H1 or CEA adducts (Fig. 3D).
levels of MGO-derived PTMs. To test this hypothesis, we developed a cell line using CRISPR-Cas technology lacking the GLO1 gene; transfection protocols are provided in Methods and Dataset S1. These cells (GLO1−/−) lacked any measurable GLO1 expression or activity (Fig. 4 A and B). GLO1−/− cells were more sensitive to MGO toxicity than wild-type (WT) cells, demonstrating a 10-fold decrease in the LC₅₀ (from 367 μM to 46 μM) following a 24-h exposure (SI Appendix, Fig. S3). This is consistent with the finding that following exposure to increasing concentrations of MGO for 1 h, GLO1−/− cells exhibited intracellular MGO concentrations nearly identical to those provided extracellularly, whereas WT cells showed no significant increase (SI Appendix, Fig. S4). When monitored over time, GLO1−/− cells displayed a significant lag-phase in MGO removal following a challenge with 50 μM MGO, and cellular concentrations returned to baseline only after approximately 12 h of exposure (SI Appendix, Fig. S5A). These findings support the accepted role of GLO1 as the primary MGO detoxifying enzyme in the case of exogenously provided MGO and illustrate its high capacity for MGO removal. Interestingly, the basal levels of MGO were not elevated in GLO1−/− cells, implying that alternative mechanisms may exist for the removal of MGO. Nevertheless, GLO1−/− cells provide a useful tool for investigating the chemical biology of MGO-derived protein adducts under conditions of elevated electrophiles, as observed in diabetes.

We next evaluated the generation of MGO-derived PTMs from total protein using QuARKMod analysis of the cell pellets isolated from the samples used in SI Appendix, Fig. S5A (21). As shown in SI Appendix, Fig. S5 B–D, GLO1−/− cells had elevated levels of MG-H1 and CEA when challenged with MGO, with concentrations peaking after approximately 6 h of exposure. CEL adduct levels were also elevated in GLO1−/− cells, reaching peak concentrations after approximately 6 h, although at approximately 10-fold lower concentrations than those of Arg-MGO adducts.

**MGO Protein Adducts Are an Abundant Chromatin PTM.** To explore the effects of elevated intracellular MGO concentrations on MGO-derived chromatin PTMs, we isolated chromatin fractions from WT and GLO1−/− cells treated for 6 h with concentrations mimicking those of the LC₅₀ (50 or 500 μM) (SI Appendix, Fig. S6). We first assessed the presence of MG-H1, MG-H2, and MG-H3/CEA in each condition via immunoblotting with antibodies directed against each isomer (22). As shown in Fig. 4 C and SI Appendix, Fig. S7, GLO1−/− cells treated with MGO were found to have marked elevations in both MG-H1 and MG-H3/CEA immunostaining, whereas MG-H2 adducts were absent.

We next applied QuARKMod to chromatin fractions to measure both canonical and MGO-derived PTMs (Fig. 4 D–G and SI Appendix, Fig. S8). Although no alterations were observed in canonical histone PTMs (Fig. 4 D and SI Appendix, Fig. S8), the levels of MG-H1, CEA, and CEL were significantly elevated in GLO1−/− cells challenged with MGO (Fig. 4 E–G). As was seen with the basal adduct levels, the CEL levels were an order of magnitude lower than Arg adduct levels.

**Identification of Site-Specific MGO Modifications on Histones.** Under basal conditions, MG-H1 and CEA are difficult to detect using standard proteomic approaches, a problem often observed with lower-abundance histone PTMs (25, 26). Attempts were made to immunopurify MGO-modified peptides from untreated samples, but these techniques yielded no enrichment. Thus, exogenous addition of MGO was required to investigate site-specific adducts. GLO1−/− cells were treated with 1 mM MGO for 6 h. This high concentration of MGO was used to generate the broadest possible range of adduction sites. Using a previously described digestion protocol, we mapped MGO adduction sites on each histone, yielding 17 MG-H adducts, 6 CEA adducts, and 5 CEL adducts (Fig. 5; the annotated MS/MS spectra for these adducts are shown in SI Appendix, Figs. S13–S40) (8). Interestingly, all the reported CEA sites were also found to contain an MG-H adduct. These data support previous findings demonstrating that the kinetic product of reaction of MGO with Arg is MG-H3, which is readily hydrolyzed to yield CEA; CEA can then recycle to yield MG-H1 (27).

Unlike many histone PTMs, MGO-derived modifications were not restricted to the heavily modified N-terminal tails; many were found on the lateral surface of the nucleosome (SI Appendix, Fig. S9A; adducts are depicted in red). H3R72, H4R23, H4R55, and H2BR92 were located in close proximity to DNA (SI Appendix, Fig. S9B). Furthermore, the presence of MG-H and CEA adducts at H3R53 (SI Appendix, Fig. S9C) may lead to disruption of nucleosomal stability akin to that of H3K56 acetylation (28).

**MGO Disrupts Global H2B Modifications.** Due to the relative abundance of MGO-derived PTMs on H3 and H2B, we hypothesized that these adducts may disrupt the formation of canonical PTMs. Consistently, MGO exposure was found to decrease H2B acetylation and ubiquitylation in GLO1−/− cells (SI Appendix, Fig. S10A); this was particularly notable in H2BK12 ubiquitylation (H2BK12Ub, H2BK12Ub is required for the binding of DOT1L, an H3K79 methyltransferase (29, 30). Despite the loss in H2BK12Ub, no alterations in H3K79me2 were observed (SI Appendix, Fig. S10A). In fact, no alterations in any canonical H3, H4, or H2A PTMs were observed (SI Appendix, Fig. S10B). These data are consistent with the hypothesis that MGO disrupts H2B PTMs, likely as a result of the high degree of modification by MGO.

**RNA-Seq Reveals Transcripts Regulated by MGO.** Histone PTMs play a critical role in the regulation of gene transcription. We therefore assessed MGO-mediated alterations in transcripts using a global RNA-Seq approach. WT or GLO1−/− cells were treated with vehicle, 50 μM MGO, or 500 μM MGO for 6 h. As shown in SI Appendix, Fig. S11 A and B, treatment of WT cells
with either 50 or 500 μM MGO had little impact on total RNA transcripts. This is likely a result of the rapid detoxification of MGO in the presence of GLO1. In GLO1−/− cells, however, a dose-dependent increase in significantly altered transcripts was observed compared with WT vehicle control (SI Appendix, Fig. S11 C–E, log2 fold change >2; P < 0.05).

We next evaluated only protein coding transcripts in the GLO1−/− cohorts with and without MGO treatment. As shown in Fig. 6, GLO1−/− cells displayed 88 significantly decreased genes and 59 elevated genes compared with WT vehicle control. Greater changes were observed following treatment with either 50 μM MGO (164 decreased and 140 increased) or 500 μM MGO (1,516 decreased and 1,281 increased) (Dataset S2). The large alterations in gene expression observed with the 500 μM MGO treatment are likely attributed to the toxicity observed with these high concentrations (greater than the LC50); therefore, we focused our attention on the 50 μM MGO cohort. Among this group, the most significantly increased genes were ADM2 (log2 fold enrichment, 3.1), POUSF2 (log2 fold enrichment, 2.3), HCL51 (log2 fold enrichment, 2.7), and SLCS6A9 (log2 fold enrichment, 2.7), and the most significantly decreased genes were FZD10 (log2 fold enrichment, −8.8), STS (log2 fold enrichment, −8.5), LTBR (log2 fold enrichment, −7.8), and EVC2 (log2 fold enrichment, −7.8).

We next performed Gene Ontology analyses using DAVID on vehicle, 50 μM MGO, and 500 μM MGO treatments to attempt to gain insight into possible pathways involved in MGO modifications (31). These analyses yielded little conclusive enrichment (Dataset S3). Notably, no enrichment was observed for antioxidant, endoplasmic reticulum stress, or heat shock responses, consistent with the hypothesis that MGO alters global transcription rather than distinct toxicity-response pathways.

**Discussion**

In this study, we identify histones as targets for modification by the glycolytic by-product MGO in intact chromatin. We report MGO-derived Arg modifications as abundant histone PTMs that are present basally and at the same order of magnitude as ADMA, a PTM involved in transcriptional regulation (23). Under conditions of high intracellular MGO, we identify 28 site-specific modifications on histones, four of which reside on the PTM-rich N-terminal tail of H3. These modifications disrupt canonical histone PTMs, most notably on H2B, where DJ-1−/− cells in both WT and GLO1−/− [double KO (DKO)] backgrounds. We then performed QuARKMod on chromatin fractions isolated from cells treated with either vehicle or 50 μM MGO for 6 h (Fig. 7 C and D). MGO treatment of DKO cells resulted in a significant increase in both MG-H1 and CEA compared with treatment of GLO1−/− cells alone. The absence of DJ-1 did not impact the levels of any Lys PTMs or ADMA (SI Appendix, Fig. S12). These data suggest a role for DJ-1 in controlling the levels of MGO-derived Arg modifications on chromatin.

**DJ-1 Reduces Histone Arg Modification by MGO.** In a recent study, Richarme et al. (32) found that the deglycase enzyme DJ-1 repaired MGO-derived modifications of guanine. DJ-1 also has been shown to prevent the formation of MGO modifications on Lys and Arg residues by hydrolyzing the aminocarbinol intermediates (Fig. 7A) (33). Therefore, we investigated the effect of DJ-1 on the levels of MGO-derived chromatin adducts. As shown in Fig. 7B, we performed CRISPR-Cas9 to generate DJ-1−/− cells in both WT and GLO1−/− [double KO (DKO)] backgrounds. We then performed QuARKMod on chromatin fractions isolated from cells treated with either vehicle or 50 μM MGO for 6 h (Fig. 7 A–C). These data suggest a role for DJ-1 in limiting the accumulation of MG-H1 and CEA on chromatin. Although the functional role of these PTMs is currently under investigation, we hypothesize that DJ-1 may serve a key regulatory signaling molecule involved in global responses due to altered glycolytic flux. This is of particular importance in the context of diabetic nephropathy, where GLO1 activity is significantly decreased and levels of MGO-modified proteins are markedly elevated (15).

**Cellular metabolism intrinsically controls gene expression and transcription through histone PTMs.** This is perhaps most notable through the acetylation of histone Lys residues via acetyl-CoA (34, 35). Many of these modifications regulate gene expression by altering chromatin structure and architecture (36). For example, acetylation at H3K56 disrupts the stability of the transcriptional apparatus, targeting the levels of MGO-derived Arg modifications on chromatin. In a recent study, Richarme et al. (32) found that the deglycase enzyme DJ-1 repaired MGO-derived modifications of guanine. DJ-1 also has been shown to prevent the formation of MGO modifications on Lys and Arg residues by hydrolyzing the aminocarbinol intermediates (Fig. 7A) (33). Therefore, we investigated the effect of DJ-1 on the levels of MGO-derived chromatin adducts. As shown in Fig. 7B, we performed CRISPR-Cas9 to generate DJ-1−/− cells in both WT and GLO1−/− [double KO (DKO)] backgrounds. We then performed QuARKMod on chromatin fractions isolated from cells treated with either vehicle or 50 μM MGO for 6 h (Fig. 7 A–C). These data suggest a role for DJ-1 in limiting the accumulation of MG-H1 and CEA on chromatin. Although the functional role of these PTMs is currently under investigation, we hypothesize that DJ-1 may serve a key regulatory signaling molecule involved in global responses due to altered glycolytic flux. This is of particular importance in the context of diabetic nephropathy, where GLO1 activity is significantly decreased and levels of MGO-modified proteins are markedly elevated (15).

**Discussion**

In this study, we identify histones as targets for modification by the glycolytic by-product MGO in intact chromatin. We report MGO-derived Arg modifications as abundant histone PTMs that are present basally and at the same order of magnitude as ADMA, a PTM involved in transcriptional regulation (23). Under conditions of high intracellular MGO, we identify 28 site-specific modifications on histones, four of which reside on the PTM-rich N-terminal tail of H3. These modifications disrupt canonical histone PTMs, most notably on H2B, where DJ-1−/− cells in both WT and GLO1−/− [double KO (DKO)] backgrounds. We then performed QuARKMod on chromatin fractions isolated from cells treated with either vehicle or 50 μM MGO for 6 h (Fig. 7 C and D). MGO treatment of DKO cells resulted in a significant increase in both MG-H1 and CEA compared with treatment of GLO1−/− cells alone. The absence of DJ-1 did not impact the levels of any Lys PTMs or ADMA (SI Appendix, Fig. S12). These data suggest a role for DJ-1 in controlling the levels of MGO-derived Arg modifications on chromatin.
The modification of proteins by MGO is reportedly the result of nonenzymatic reactions with Arg and Lys residues; however, our data suggest that these modifications are regulated by eraser proteins. In support of Richarme et al. (33), we demonstrate that DJ-1 “protects” Arg residues from modification by MGO, while not impacting other canonical PTMs. Although no increases MG-H1 or CEA were observed in the DJ-1+/− cells, this is likely due to the rapid metabolism of MGO in cells expressing GLO1. Furthermore, these studies support a recent report that defined a critical role for DJ-1 in maintaining DNA integrity via the regulation of gene expression, rather than simply serving as a reactive molecule responsible for the induction of electrophile-response pathways (40).

The modification of proteins by MGO is reportedly the result of nonenzymatic reactions with Arg and Lys residues; however, our data suggest that these modifications are regulated by eraser proteins. In support of Richarme et al. (33), we demonstrate that DJ-1 “protects” Arg residues from modification by MGO, while not impacting other canonical PTMs. Although no increases MG-H1 or CEA were observed in the DJ-1+/− cells, this is likely due to the rapid metabolism of MGO in cells expressing GLO1. Furthermore, these studies support a recent report that defined a critical role for DJ-1 in maintaining DNA integrity via the regulation of gene expression, rather than simply serving as a reactive molecule responsible for the induction of electrophile-response pathways (40).

The modification of proteins by MGO is reportedly the result of nonenzymatic reactions with Arg and Lys residues; however, our data suggest that these modifications are regulated by eraser proteins. In support of Richarme et al. (33), we demonstrate that DJ-1 “protects” Arg residues from modification by MGO, while not impacting other canonical PTMs. Although no increases MG-H1 or CEA were observed in the DJ-1+/− cells, this is likely due to the rapid metabolism of MGO in cells expressing GLO1. Furthermore, these studies support a recent report that defined a critical role for DJ-1 in maintaining DNA integrity via the regulation of gene expression, rather than simply serving as a reactive molecule responsible for the induction of electrophile-response pathways (40).

The modification of proteins by MGO is reportedly the result of nonenzymatic reactions with Arg and Lys residues; however, our data suggest that these modifications are regulated by eraser proteins. In support of Richarme et al. (33), we demonstrate that DJ-1 “protects” Arg residues from modification by MGO, while not impacting other canonical PTMs. Although no increases MG-H1 or CEA were observed in the DJ-1+/− cells, this is likely due to the rapid metabolism of MGO in cells expressing GLO1. Furthermore, these studies support a recent report that defined a critical role for DJ-1 in maintaining DNA integrity via the regulation of gene expression, rather than simply serving as a reactive molecule responsible for the induction of electrophile-response pathways (40).

Methods

More detailed information on the study methodology is provided in SI Appendix, Methods.

Cell Culture. HEK293 cells were cultured in low-glucose DMEM (1 g/L glucose) supplemented with 10% FBS. Cells were incubated at 37 °C and under 5% CO2. SILAC cells were cultured in low-glucose SILAC medium supplemented with 0.1 g/L 13C2,15N2 Lys, 0.1 g/L 13C6,15N6 Arg, 0.1 g/L 13C3,15N Leu for “heavy” cells or natural abundance isotopes for “light” cells, and 10% dialyzed FBS (Fisher Scientific). Isotope incorporation was monitored at each passage via QuARKMod and labeling was considered complete when incorporation exceeded 95%.

Generation of GLO1+/− and DJ-1+/− Cells Using CRISPR-Cas9. gRNA oligonucleotides (Dataset S1) were designed to target restriction enzyme recognition sites in the initial exons of the GLO1 or DJ-1 locus and ligated into the pSpCas9(BB)-2A-Puro plasmid as described by Cong et al. (41). To generate cells lacking GLO1 or DJ-1, 2 × 105 HEK293 cells were plated in 2 mL of DMEM supplemented with 10% FBS in six-well plates. The next day, 5 μg of each construct was combined with 10 μL of Lipofectamine 2000 reagent (Life Technologies) in 1 mL of Opti-MEM and incubated at room temperature for 30 min. The DMEM was replaced with the small-molecule-Lipofectamine 2000 solution, and the cells were incubated at 37 °C for 24 h. The medium was replaced, after which the cells were allowed to recover for 24 h at 37 °C. The medium was then replaced with serum-containing DMEM with 0.75 μg/mL puromycin, and the cells were incubated at 37 °C for 48 h. The medium was then replaced with puromycin-free medium, and the cells were incubated for another 24 h, after which the medium was replaced to remove any traces of puromycin. Cultures were then pelleted, resuspended in sorting buffer (PBS + 4% FBS), and stained. Solutions were sorted by flow cytometry using a BD FACSAria III cell sorter to isolate single cells. Cells were maintained to approximately 80% confluency and passaged until a sufficient number of cells could be harvested for analysis. A restriction-fragment length polymorphism (RFLP) assay (SI Appendix, Table S1) was used to assess for indel mutations; target genes were PCR-amplified and subjected to digestion by restriction enzymes.
specific to the WT sequence at the gRNA target site. The absence of restriction enzyme activity was indicative of mutations in both alleles at the gRNA target site, and clones with homozygous mutations of GLO1 or DJ-1 were validated as genetic knockouts. PCR products from RFLP assays were purified using the Nucleospin PCR Clean-Up Kit and sequenced with the forward primer (Dataset S1). Sequencing samples were analyzed by GenHunter Corporation.

ACKNOWLEDGMENTS. We thank William P. Tansey and Carol A. Rouzer for their thoughtful discussions and input. Financial support was provided by National Institutes of Health Grants CA87819 and S10 OD017997 (to L.J.M.) and the SENS Foundation (to D.A.S.). The Vanderbilt University Medical Center Flow Cytometry Shared Resource is supported by the Vanderbilt Ingram Cancer Center (Grant P30 CA68485) and the Vanderbilt Digestive Disease Research Center (Grant DK58404). Vanderbilt Technologies for Advanced Genomics (Vantage) is supported in part by the Clinical and Translational Science Awards Program (Grant 1UL1TR002371-01), the Vanderbilt Ingram Cancer Center (Grant P30 CA68485), the Vanderbilt Vision Center (Grant P30 EY08126), and National Center for Research Resources (Grant G20 RR030956). Supplemental funding to Vantage and Vanderbilt Technologies for Advanced Genomics Analysis and Research Design is provided by National Institutes of Health Grants P50 GM115305 and U19 HL065962.

1. Jenuwein T, Allis CD (2001) Translating the histone code. Science 293:1074–1080.
2. Huang H, Sabari BR, Garcia BA, Allis CD, Zhao Y (2014) SnapShot: Histone modifica-
tion technologies for advanced genomics analysis and research design is provided
3. Fan J, Krautkramer KA, Feldman JL, Denu JM (2015) Metabolic regulation of histone
post-translational modifications. ACS Chem Biol 10:95–108.
4. Sabari BR, Zang D, Allis CD, Zhao Y (2017) Metabolic regulation of gene expression
forward primer (Dataset S1). Sequencing samples were analyzed by GenHunter Corporation.
5. Galligan JT, Marnett LJ (2017) Histone adduction and its functional impact on
epigenetics. Chem Rev 110:376–387.
6. Jiang T, Zhou X, Taghizadeh K, Dong M, Dedon PC (2007) N-formylation of lysine in
histone proteins as a secondary modification arising from oxidative DNA damage.
Proc Nat Acad Sci USA 104:60–65.
7. Wisniewski JR, Zougman A, Mann M (2008) N-epsinil-formylation of lysine is a
widespread post-translational modification of nuclear proteins occurring at residues
involved in regulation of chromatin function. Nucleic Acids Res 36:570–577.
8. Galligan JJ, et al. (2015) Stable histone adduction by 4-oxo-2-nonenal: A potential link
between oxidative stress and epigenetics. J Am Chem Soc 136:11864–11866.
9. Cui Y, Li X, Lin J, Hao Q, Li XD (2017) Histone ketoamide adduction by 4-oxo-2-
nonenal is a reversible posttranslational modification regulated by Sirt2. ACS Chem Biol 12:47–51.
10. Jin J, He B, Zhang X, Lin H, Wang Y (2016) SIRT2 reverses 4-oxononanoyl lysine
modification on histones.
11. Sola-Penna M, Da Silva D, Coelho WS, Marinho-Carvalho MM, Zancan P (2010) Reg-
ulation of mammalian mammalian muscle type 6-phosphofructo-1-kinase and its implication
for the control of the metabolism. JUBMB Life 62:791–796.
12. Moelling RE, Cravatt BF (2014) Exploring post-translational arginine modifi-
cations using chemically synthesized methylglyoxal hydroimidazolones. J Am Chem
Soc 136:8958–8967.
13. Zhou L, et al. (2016) Evidence that ubiquitylated H2B corrals hDot1L on the nucleo-
somal surface to induce H3K79 methylation. Nat Commun 7:10589.
14. Whitcomb SJ, et al. (2012) Histone monoubiquitylation position determines specificity
and direction of enzymatic cross-talk with histone methyltransferases Dot1L and
PRC1. J Biol Chem 287:23718–23725.
15. Huang W, Sherman BT, Lenzicki RA (2009) Systematic and integrative analysis of
large gene lists using DAVID bioinformatics resources. Nat Protoc 4:44–57.
16. Richarne G, et al. (2015) Parkinsonism-associated protein DJ-1/Park7 is a major pro-
gtein degracyte that repairs methylglyoxal- and glyoxal-glycated cysteine, arginine,
and lysine residues. J Biol Chem 290:1885–1897.
17. Zheng Y, Thomas PM, Kelleher NL (2013) Measurement of acetylation turnover at
distinct lysines in human histones identifies long-lived acetylated sites. Nat Commun
4:2203.
18. Galli G, et al. (2012) Quantitative analysis and discovery of lysine and arginine
modifications. Anal Chem 89:1299–1306.
19. Wang T, Streeter MD, Spiegel DA (2015) Generation and characterization of anti-
bodies against arginine-derived advanced glycation end products. Bioorg Med Chem
Lett 25:4881–4886.
20. Agalou S, Ahmed N, Babaei-Jadidi R, Dawnay A, Thornalley PJ (2005) Profound mis-
regulation of gene expression and chromatin structure occurs in nondiabetic women.
J Biol Chem 280:565–668.
21. Galligan JJ, et al. (2016) Quantitative analysis and discovery of lysine and arginine
modifications. Anal Chem 89:1299–1306.
22. Wang T, Kartika R, Spiegel DA (2012) Exploring post-translational arginine modifi-
cation using chemically synthesized methylglyoxal hydroimidazolones. J Am Chem
Soc 134:4219–4224.
23. Tessarz P, Kouzarides T (2014) Histone core modifications regulating nucleosome
structure and dynamics. Nat Rev Mol Cell Biol 15:703–708.
24. Zhou L, et al. (2016) Evidence that ubiquitylated H2B corrals hDot1L on the nucleo-
somal surface to induce H3K79 methylation. Nat Commun 7:10589.
25. Whitcomb SJ, et al. (2012) Histone monoubiquitylation position determines specificity
and direction of enzymatic cross-talk with histone methyltransferases Dot1L and
PRC1. J Biol Chem 287:23718–23725.
26. Huang W, Sherman BT, Lenzicki RA (2009) Systematic and integrative analysis of
large gene lists using DAVID bioinformatics resources. Nat Protoc 4:44–57.
27. Richarne G, et al. (2015) Parkinsonism-associated protein DJ-1/Park7 is a major pro-
gtein degracyte that repairs methylglyoxal- and glyoxal-glycated cysteine, arginine,
and lysine residues. J Biol Chem 290:1885–1897.
28. Zheng Y, Thomas PM, Kelleher NL (2013) Measurement of acetylation turnover at
distinct lysines in human histones identifies long-lived acetylated sites. Nat Commun
4:2203.
29. Galli G, et al. (2012) Quantitative analysis and discovery of lysine and arginine
modifications. Anal Chem 89:1299–1306.
30. Wang T, Streeter MD, Spiegel DA (2015) Generation and characterization of anti-
bodies against arginine-derived advanced glycation end products. Bioorg Med Chem
Lett 25:4881–4886.
31. Fuhrmann J, Thompson PR (2016) Protein arginine methylation and citrullination
in epigenetic regulation. J Biol Chem 291:23725–2318.
32. Nokini M, et al. (2017) Hormetic potential of methylglyoxal, a side-product of gly-
colysis, in switching tumours from growth to death. Sci Rep 7:11722.
33. Huang H, Lin S, Garcia BA, Zhao Y (2015) Quantitative proteomic analysis of histone
modifications. Chem Rev 115:2376–2418.
34. Larsen SC, et al. (2016) Proteome-wide analysis of arginine monomethylation reveals
widespread occurrence in human cells. Sci Signal 9:rs9.
35. Galdieri L, Vancura A (2012) Acetyl-CoA carboxylase regulates global histone acety-
lation. EMBO Rep 13:650–656.
36. Grunstein M (1997) Histone acetylation in chromatin structure and transcription.
Nat Rev Mol Cell Biol 8:95–108.
37. Richarne G, et al. (2015) Parkinsonism-associated protein DJ-1/Park7 is a major pro-
gtein degracyte that repairs methylglyoxal- and glyoxal-glycated cysteine, arginine,
and lysine residues. J Biol Chem 290:1885–1897.
38. Zheng Y, Thomas PM, Kelleher NL (2013) Measurement of acetylation turnover at
distinct lysines in human histones identifies long-lived acetylated sites. Nat Commun
4:2203.