Nuclear phosphatidylinositol-5-phosphate regulates ING2 stability at discrete chromatin targets in response to DNA damage

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ING2 (inhibitor of growth family member 2) is a component of a chromatin-regulatory complex that represses gene expression and is implicated in cellular processes that promote tumor suppression. However, few direct genomic targets of ING2 have been identified and the mechanism(s) by which ING2 selectively regulates genes remains unknown. Here we provide evidence that direct association of ING2 with the nuclear phosphoinositide phosphatidylinositol-5-phosphate (PtdIns(5)P) regulates a subset of ING2 targets in response to DNA damage. At these target genes, the binding event between ING2 and PtdIns(5)P is required for ING2 promoter occupancy and ING2-associated gene repression. Moreover, depletion of PtdIns(5)P attenuates ING2-mediated regulation of these targets in the presence of DNA damage. Taken together, these findings support a model in which PtdIns(5)P functions as a sub-nuclear trafficking factor that stabilizes ING2 at discrete genomic sites.

Chromatin structure plays a central role in the establishment, maintenance, and propagation of nuclear programs and the dysregulation of gene expression programs occurs in numerous human pathologies, including cancer. A key mechanism regulating chromatin structure involves the reversible covalent post-translational modification (PTM) of histone proteins by chemical moieties such as acetyl- and methyl-groups. Histone PTMs are dynamic, frequently changing in response to external cues1–3. For example, histone PTMs acutely change during DNA damage responses (DDRs)4–6. A key question in understanding how nuclear programs, such as DDRs, are appropriately orchestrated is to identify how chromatin-regulatory activities are specifically targeted and maintained at discrete chromosomal sites.

Inhibitor of Growth family member 2 (ING2) is a tumor suppressor protein7 that is a core component of a multi-factor chromatin-modifying complex containing the transcriptional co-repressor SIN3A and HDAC1 (histone deacetylase 1)8. In response to DNA damage, ING2 associates with chromatin where it modulates crosstalk between lysine methylation and lysine acetylation on histone proteins9. ING2 is stabilized at active chromatin via its PHD (Plant homeodomain) finger9, which selectively binds to H3K4me3 (Histone H3 trimethylated on lysine 4), a hallmark of active transcription11,12. Genomic profiling of H3K4me3 patterns in a variety of cell types conclude that H3K4me3 levels are high in the 5′ region of nearly all actively expressed genes and, consistently, H3K4me3 promoter enrichment correlates with active RNA polymerase occupancy, and histone hyper-acetylation13. Thus, the interaction between ING2 and H3K4me3 (ING2::K4me3) stabilizes ING2-SIN3A-HDAC1 complexes at target gene promoters, regions rich in acetylated histones, resulting in histone deacetylation and gene repression9,14. However, the mechanism(s) that senses DNA damage and places ING2 in the vicinity of H3K4me3-rich target gene promoters is not known.

Phospholipids, including phosphoinositides (PtdInsPs) play a pivotal role in cytoplasmic trafficking of intracellular proteins15,16. While relatively little is known about the roles of nuclear PtdInsPs compared to their cytosolic counterparts, recent evidence suggests that nuclear phosphoinositides control the localization of proteins in the nucleus17–21. The direct interaction between ING2 and nuclear phosphatidylinositol-5-phosphate (PtdIns(5)P) changes sub-nuclear distribution of ING2, which facilitates recruitment of ING2 to chromatin, and...
is required for ING2-associated activation of p53 during DDRs. Interestingly, levels of PtdIns(5)P undergo dynamic fluctuations within the nucleus during DDRs. For example, etoposide, a topoisomerase inhibitor, increases the level of PtdIns(5)P in the chromatin of human fibrosarcoma cells (HT1080). These observations suggest a model in which dynamic changes in PtdIns(5)P levels could modulate selective chromatin targeting of ING2.

Here we demonstrate that the interaction between ING2 and nuclear PtdIns(5)P (ING2::PtdIns(5)P) directly regulates chromatin-mediated gene expression control. We first identify the DNA damage-associated gene expression network mediated by ING2 and then uncover the subset of these genes that are specifically modulated by association of ING2 with PtdIns(5)P. For this cluster, there is a loss of ING2-associated gene repression when ING2::PtdIns(5)P is disrupted or when nuclear PtdIns(5)P is metabolized. Loss of ING2 promoter occupancy is concomitant with abrogation of DNA damage-induced histone deacetylation occurring in aborting transcript elevation. Taken together, these findings establish PtdIns(5)P as a new class of nuclear signaling molecules controlling gene expression and provide mechanistic insight into the role of sub-nuclear trafficking in the acute regulation of chromatin structure.

Results
Characterization of an ING2-associated DNA damage gene expression program. To test the role of PtdIns(5)P-binding by ING2 on ING2 gene expression functions, we determined the ING2-mediated DNA damage program using a comprehensive genomic approach coupling ChIP-chip (chromatin immunoprecipitation combined with tiling promoter DNA microarrays) and gene expression microarray analyses. Combining these assays allowed for the identification of gene promoters bound by ING2 as well as the downstream transcriptional changes. Once we established a baseline, we monitored changes to the ING2 expression network resulting from perturbations in PtdIns(5)P signaling (diagrammed in Figure S1).

For this study it was necessary to identify a form of ING2 that does not bind PtdIns(5)P, but is not deficient in H3K4me3 binding. The C-terminus of ING2 (Figure 1A) contains two conserved modules (1) a PHD finger and (2) a polybasic region (PBR); within these modules several key residues have been characterized for their role in histone or lipid binding (see Figure 1B). For example, a specific single amino acid change within the PHD finger (ING2D230A) abrogates ING2::H3K4me3 without ablation of ING2::PtdIns(5)P. Here we tested if a previously identified mutant deficient in PtdIns(5)P binding (named ING2D230A) can bind H3K4me3. Additionally we generated and characterized a mutant with the combined amino acid changes in ING2D230A and ING2D230A named ING2D-6K/Rmt. We subjected the panel of ING2 mutants to in vitro histone peptide (Figure 1C) and liposome pull-down assays (Figure 1D). As shown in Figure 1C, ING2D230A which fails to bind to PtdIns(5)P-containing liposomes, binds to H3K4me3 comparable to wild-type. In contrast, ING2D-6K/Rmt is deficient in binding to both H3K4me3 and PtdIns(5)P (Figure 1D). Thus ING2D-6K/Rmt and the other ING2 mutants can be used to isolate the impact of the ING2::PtdIns(5)P on ING2 gene-regulatory functions. Next we generated human fibrosarcoma cells (HT1080) stably-expressing epitope-tagged, full-length wild-type ING2 (ING2WT) or ING2D-6K/Rmt. Note that endogenous ING2 protein levels are depleted in response to ectopic expression of wild-type and mutant ING2, thus the majority of ING2 in the reconstituted cells is exogenous (Figure 1E; data not shown).

Next, genome-wide promoter occupancy of ING2WT was determined. DNA that co-purified with FLAG-ING2WT in vehicle-treated and etoposide-treated HT1080 cells was hybridized to Nimblegen whole genome human promoter arrays and peaks were detected using Nimblescan software. Significant ING2 peaks (see Methods) were identified at over 200 unique promoters in etoposide-treated cells, whereas no significant ING2 peaks were detected in vehicle-treated cells (Supplementary Table 1). We hereafter refer to the set of genes selectively bound by ING2 in the presence of etoposide as ING2ET-BOUND targets.

Heatmap displays and average binding profiles were generated to highlight ING2 occupancy within the promoters of ING2ET-BOUND genes (Figure 2A; Figure S2). ING2 is enriched in promoters both upstream and downstream of the transcriptional start site (TSS) with decreased occupancy in the nucleosome-poor TSS, this is consistent with the known distribution of H3K4me3 dictating ING2 occupancy via the ING2::H3K4me3 interaction. Next we tested if endogenous ING2 behaves the same as FLAG-tagged ING2 at a novel ING2 target we identified: DKC1 (Figure 2B). The FLAG-ING2 ChIP-chip data (Figure 2B top) is consistent with direct ChIP assays of endogenous ING2 (Figure 2B bottom): ING2 associates with the DKC1 promoter in the presence of etoposide with strong enrichment upstream of the TSS. Collectively, we identified a cluster of over two hundred novel gene promoters that ING2 selectively associates with in the presence of DNA damage.

To determine if etoposide-induced ING2 occupancy results in a functional change in transcription at ING2 target genes we performed a series of gene expression microarray experiments. Note that promoter occupancy was assayed 1 hr after etoposide treatment, while gene expression analyses were monitored 20 hrs after etoposide treatment to allow for transcriptional responses to occur. Global transcript profiling indicated that ING2ET-BOUND genes (Cluster A) represent a statistically-significant (p = 3.3E-11, Fisher’s exact test) population of genes repressed in response to etoposide (Figure 2C, top). Consistently, both of these gene clusters contain enrichment of factors that modulate chromosome organization and the cell cycle (Figure 2C, bottom). To further explore the relationship between ING2 promoter occupancy and transcriptional repression we tested more promoter targets. Endogenous ING2 is promoter-bound at AURKB (a representative from the cell cycle cluster) and Pcgf2 (a representative from the chromosome organization cluster), but not at ACTA, a negative control (Figure 2D). ING2 occupancy correlates with a decrease in acetylated H3 at lysine 27 (H3K27Ac; Figure 2E). These data are consistent with ING2 being present in an HADC1 complex. Taken together, these findings support the notion that ING2 plays a key role in DNA damage-induced gene repression, and this activity may be critical for ING2 tumor suppressive functions.

The ING2-PtdIns(5)P interaction is required for execution of the ING2-associated DNA damage gene expression program. Based on the observations that (1) ING2 dynamically associates with chromatin in response to etoposide to repress transcription, and (2) nuclear PtdIns(5)P levels increase in response to etoposide treatment, we postulated that DDR-induced ING2-PtdIns(5)P interactions would regulate ING2 occupancy and/or activity at target genes. To test this we performed global gene expression analyses on cells expressing ING2D230A (a representative from the chromosome organization cluster) and in vehicle-treated, whereas no significant ING2 peaks were detected in vehicle-treated cells (Supplementary Table 1). We hereafter refer to the set of genes selectively bound by ING2 in the presence of etoposide as ING2ET-BOUND targets.

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Figure 1 | ING2 independently interacts with H3K4me3 and PtdIns(5)P. (A) (Top) Coomassie stain of GST-fused ING2 C-terminus constructs. (Bottom) Schematic showing protein domain organization in the ING2 constructs. Asterisk indicates site of mutagenesis in which the endogenous amino acid is replaced with alanine: in the PHD domain D230A; in the PBR domain K272A, K274A, K275A, R277A, R278A, and R280A. (B) Table summarizing GST-ING2 fusion binding activities. (C) Biotinylated histone pull-downs for ING2 constructs. (D) Liposome pull-downs for ING2 constructs. (E) Immunoblot panel of HT1080 cell systems constitutively expressing ectopic ING2 forms. Uncropped images of blots are shown in Supp Figure 3.
Figure 2 | ING2 is a critical mediator of chromatin dynamics in the presence of DNA damage. (A) Dynamic association of ING2 with gene promoters in response to etoposide. Average ING2 promoter enrichment in ChIP-chip assays (two replicates of Etoposide (ET)-treated cells, two replicates of vehicle (V)-treated cells). (B) Direct ChIP of endogenous ING2 is consistent with FLAG-ING2 ChIP-chip determined promoter occupancy. (Top) ING2 enrichment at the DKC1 promoter in the indicated ChIP-chip experiments. (Bottom) Direct ChIP of endogenous ING2 at the promoter of DKC1 under conditions indicated. Control = no antibody control IP. (C) Enrichment of ING2ET-BOUND genes in the set of transcripts repressed in response to DNA damage. (Top) Venn diagram highlighting the overlap between genes directly regulated by ING2 and genes repressed in response to etoposide. p-value indicates significance of overlap determined by Fisher’s exact test. (Bottom) Gene Ontology (GO) analyses of ING2ET-BOUND genes (Cluster A) or Etoposide-repressed genes (Cluster B). (D) ChIP of endogenous ING2 at the promoters of Actin (ACTA), Aurora Kinase B (AURKB), and Polycomb group RING finger protein 2 (PCGF2) in etoposide-treated cells. Control = no antibody control IP. (E) ChIP of Histone H3 acetylated at Lysine 27 (H3K27Ac) at the AURKB and PCGF2 promoters in the absence and presence of etoposide. See also Supp Figure 2 and Supp Table 1.
Figure 3 | The ING2::PtdIns(5)P interaction is a critical modulator of the nuclear program mediated by ING2. (A) Reprogramming of transcriptional repression in cells lacking ING2-PtdIns(5)P. Venn diagram highlighting the overlap between genes repressed in response to etoposide in cells expressing ING2wt or ING26K/Rmt. (B) Massive impairment of Cell Cycle components when the ING2-PtdIns(5)P interaction is ablated. Comparison of gene expression changes of Cell Cycle modulators (Cluster B in Figure 2C) in cells expressing ING2wt or ING26K/Rmt. (C) The transcript level of 5PRESS ING2 targets (AURKB and SKP2) are elevated in cells lacking the ING2::PtdIns(5)P interaction. The mRNA levels (determined by qPCR) of ING2 Etoposide-bound targets: AURKB, DKC1, SKP2, and UBE2N. (D) Full-length ING26K/Rmt purified from cells associates with comparable HDAC activity as ING2wt. (Top) Immunoblot analysis of FLAG immunoprecipitates from HT1080 populations stably-expressing: ING2wt, ING2D230A, ING26K/Rmt, ING2D-6K/Rmt, or empty vector (control). (Bottom) Immunoblot analysis of in vitro HDAC reactions on bulk, purified histones. Where indicated, reactions were performed in the presence of trichostatin A (TSA). Uncropped images of blots are shown in Supp Figure 4.
cells. Hereafter this expression pattern is called 5PRESS (transcripts that require the binding event between ING2 and PtdIns(5)P for repression in response to etoposide). Quantitative PCR transcript (qPCR) analyses were performed on several ING2ET-BOUND targets to confirm the 5PRESS expression signature (Figure 3C). In cells treated with etoposide the transcript level of UB2EN and DKK1 are similar in cells ectopically expressing ING2WT or ING26K/Rmt. In contrast, the transcript levels of SKP2 and AURKB (5PRESS targets) are elevated over 5-fold in cells expressing ING26K/Rmt compared to cells expressing ING2WT. To summarize, transcript analyses indicate that inhibition of the ING2-PtdIns(5)P interaction results in aberrant DDR-induced gene repression.

One possible explanation for the defect in gene repression of cells expressing ING26K/Rmt is that this mutant has decreased association with HDAC activity. To investigate this possibility, a panel of ING2 complexes were purified then tested for in vitro HDAC activity (Figure 3D). Compared to ING2WT, affinity-purified ING26K/Rmt associated with comparable levels of HDAC1, SAP30, and SIN3A (Figure 3D, top). Furthermore, affinity-purified ING26K/Rmt exhibits in vitro histone deacetylase (HDAC) activity (on H3K9 and H3K27) comparable to ING2WT (Figure 3D, bottom). We conclude that ablation of ING2::PtdIns(5)P does not alter ING2-associated HDAC activity. Taken together, these results strongly argue a direct role for PtdIns(5)P in mammalian gene regulation.

PtdIns(5)P is required for stabilization at a spectrum of ING2 promoter targets. To focus functionally on the role of PtdIns(5)P in selective gene repression we narrowed our attention to genes directly bound by ING2 that require ING2::PtdIns(5)P for repression (hereafter called ING25PRESS-BOUND; Figure 4A). More specifically, ING25PRESS-BOUND targets: (1) are ING2 ET-BOUND, specifically, ING2 5PRESS-BOUND targets: (1) are ING2 ET-BOUND, (2) have the 5PRESS expression signature. PtdIns(5)P modulates repression of ~10% of ING2 direct targets, note the remaining ING2 targets are hereafter referred to as ING2CONTROL-BOUND (Figure 4A). This group of genes is involved in diverse cellular processes such as cell cycle control, organelle homeostasis, and RNA processing (Figure 4A). Intriguingly, the ING2-PtdIns(5)P interaction is required for repression of CIP2A (also known as KIAA1524), a putative oncogene that promotes anchorage-independent growth31.

Our working model was that select ING2 targets require stable association of ING2 with PtdIns(5)P to properly traffic ING2 to the promoter (Figure 4B). To test our model we determined if ablation of ING2::PtdIns(5)P would result in loss of ING2 promoter targeting. Promoter occupancy for the ING2 panel was tested at an ING25PRESS-BOUND representative, CIP2A, as well as an ING2CONTROL-BOUND representative, UB2EN, in which ING2 promoter occupancy should not be regulated by PtdIns(5)P (Figure 4C). ING26K/Rmt associates with the promoter of UB2EN, but is not promoter-bound at CIP2A. Furthermore, neither ING26K/Rmt nor ING2D-6K/Rmt are enriched compared to the control IP (FLAG-IP from mock-transduced cells), which is consistent with the critical role of H3K4me3::ING2 interaction in stabilizing ING2 at target promoters. These data support the hypothesis that ING2::PtdIns(5)P is required for promoter occupancy at discrete ING2 chromatin targets.

Next we determined if chromatin trafficking of endogenous ING2 is impaired in an established cell-based system in which PtdIns(5)P signaling is depleted due to over-expression of the human enzyme phosphatidylinosititol 4-phosphate kinase II beta (PIP4Kbeta), which utilizes PtdIns(5)P as a substrate to generate PtdIns(4,5)P2. PIP4Kbeta expression abrogates association of endogenous ING2 with the ING25PRESS-BOUND representative, CIP2A, but not with the ING2CONTROL-BOUND representative, UB2EN (Figure 4D top). Furthermore in PIP4Kbeta-expressing HT1080 cells, acetylated histone H4 (Acetyl-H4) levels are elevated exclusively at the CIP2A promoter (Figure 4D bottom). These promoter changes have a functional consequence on gene expression since ectopic PIP4Kbeta results in a loss of DDR-induced gene repression for CIP2A, but not UB2EN or PGK1, a gene not directly regulated by ING2 (Figure 4E). Thus, disrupting the interaction between ING2 and PtdIns(5)P by two independent methods impairs the ability of ING2 to bind and subsequently repress transcription of a subset of ING2 target genes.

Discussion
An understanding of the gene network controlled by ING2 is critical to unravel the molecular mechanism by which ING2 functions under physiological conditions as a tumor suppressor protein. Here, we uncovered several key features of the nuclear program mediated by ING2 in response to DNA damage. We identified more than 200 novel gene promoters directly bound by ING2 in response to genotoxic stress. Consistent with tumor suppressive functions, ING2 regulates several key cellular processes including chromatin structure, cell cycle progression, and cell proliferation. We focused on a group of ING2 targets that are dependent on the nuclear lipid PtdIns(5)P for proper regulation. The genes controlled by PtdIns(5)P are dysregulated when either (1) the binding event between ING2 and PtdIns(5)P is abrogated or (2) PtdIns(5)P levels are depleted due to PIP4Kbeta over-expression. Together, our data suggest that PtdIns(5)P acts to localize ING2 to target gene promoters which is instrumental in focusing ING2-associated HDAC activity. PtdIns(5)P was discovered nearly two decades ago32, and our work provides a new molecular mechanism of how PtdIns(5)P can function in the context of signal transduction within the nucleus.

Chromatin and phospholipid signaling networks impact gene expression and dysregulation of either one of these networks can contribute to human pathological states including cancer. ING2 is of great interest because it is a direct integrator of chromatin and nuclear phosphoinositide signaling networks and here we report that these pathways converge to regulate the putative oncogene CIP2A. Our data favor a model in which the ING2::PtdIns(5)P interaction stabilizes ING2 at discrete sites within the nucleus, where ING2 can then bind to H3K4me3 and anchor ING2-HDAC1-SIN3 complexes at gene promoters. While this study identifies a specialized role for ING2::PtdIns(5)P in gene expression control, it also highlights that ING2::H3K4me3 may have a more general role in gene regulatory functions. In Figure 4C we find that the mutant of ING2 unable to bind H3K4me3 is not bound at either ING2 direct target. While histone PTMs clearly have important roles in the stabilization and maintenance of regulatory complexes on the chromatin template, selective control of gene expression requires a large repertoire of factors including DNA sequence-specific factors and other regulatory cofactors32. Here we present evidence that nuclear phosphoinositides may participate as regulatory cofactors playing a critical role in chromatin dynamics. In contrast to the numerous chromatin-binding domains that have been characterized, few predominantly nuclear lipid-binding proteins have been characterized. However, a recent report elaborately employed a proteomic approach to identify over one hundred nuclear proteins that interact with the phosphoinositide PtdIns(4,5)P2. We speculate that follow-up studies on some of these candidates, as well as discovery of additional PtdIns(5)P-effectors, will uncover the diversity of functions of nuclear PtdInsPs in gene regulation and other fundamental nuclear processes.

Methods
Histone-peptide and liposome binding assays. Biotinylated H3 peptides and histone-peptide binding assays have been described previously33. Lipids were purchased from Echelon biosciences or Avanti Polar lipid. Liposome binding assays
**Figure 4 | Select ING2 targets require PtdIns(5)P for promoter stabilization.** (A) ING2 direct targets that require ING2-PtdIns(5)P for DNA damage-induced repression (5PRESS-BOUND) are involved in diverse processes. Rep = Biological replicate. (B) Schematic of model in which ING2ET-BOUND genes are divided into two groups. Predicted effect of PIP4Kbeta over-expression on ING2 target gene regulation is highlighted. (C) ING26K/Rmt is promoter-bound at UBE2N, but not promoter-bound at CIP2A. Relative anti-FLAG ING2 ChIP from HT1080 cell lines above treated with etoposide (100 uM) for 1 hr. (D) Over-expression of PIP4Kbeta results in decreased ING2 occupancy as well as increased acetylation of histone H4 at the 5PRESS-BOUND representative, CIP2A. Direct ChIP was performed with indicated antibodies in cells treated with etoposide. Data represent averages of 3 independent experiments. * p = 4.86E-02, ** p = 4.43E-02, one-tailed Student’s t-test. (E) Ectopic expression of PIP4Kbeta impairs ING2-mediated repression in the presence of etoposide. mRNA transcript levels of CIP2A are aberrantly elevated with little effect on UBE2N or PGK1 in cells expressing PIP4Kbeta. Data represent averages of 3 independent experiments.
were carried out essentially as described in. Briefly, recombinant protein and liposomes were combined in binding buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 0.05% Nonidet P-40), rotated for 1 hr at room temperature, and then centrifuged at 13,000 rpm for 2 min. The liposome pellet was washed three times in 1 ml of binding buffer. Liposome pellets were resolved by SDS-PAGE, and GST-fusion proteins were detected by Immunoblot using an anti-GST antibody (Abcam).

ChIP and ChIP-chip. HT1080 cells were treated with Etoposide (100 μM) or vehicle (DMSO) for 1 hr, then prepared as described previously for chromatin immunoprecipitation (ChIP)11. Purified ChIP DNA was quantified by real-time PCR (RT-PCR) using a LightCycler 480 II (Roche) or a 7300 Real Time PCR machine (Applied Biosystems). Primers are listed in Supp Table 3. For direct ChIP experiments (unless otherwise indicated), a representative experiment is shown with error bars indicating standard error of the mean (s.e.m.) of three technical replicates. ChIP-chip assays were performed as previously described10. Briefly, ChIP DNA purified as described above, then amplified (Sigma Whole Genome Amplification kits 2 and 3), and labeled, and hybridized to Roche Nimblegen 385 K ReSeq promoter arrays. ChIP-chip peaks were identified using the MACS algorithm as described in the manufacturer’s protocol. A peak must have a minimum of four consecutive ChIP-chip probes where the log2 ratio of ChIP to input signal is between 20-90% of the hypothetical maximum log2 ratio (the mean log2 ratio of the array + 6 standard deviations). The false discovery rate (FDR) for each peak was calculated by permutations of the data within each chromosome, our cutoff was FDR less than or equal to 0.2. We applied an additional cutoff which required the highest four probe peaks within the peak to have a log2 ratio > 0.5 (ING2 ChIP/input chromatin). To be a significant ING2 peak, the gene had to meet all of the criteria above in both biological replicates.

Antibodies and Plasmids. ING2 and derivative constructs were previously described11,12. PIP4K beta and derivative constructs were previously described or subcloned into: pBabe with FLAG-HA epitopes12,13. Mutants were made by PCR-mediated site-directed mutagenesis (Stratagene). Antibodies used in this study: ING222, H3K4me3 (Active Motif); SAP30, HDAC1 and Acetyl-H4 (Millipore); Rabbit IgG, FLAG M2 and H3Kac (Sigma).

Transcript analyses. RNA was isolated using RNeasy plus kits (Qiagen). For gene expression microarrays, RNA was processed for use with Illumina Bead Arrays per manufacturer’s instructions. Probe-level data were processed using Genespring software and all subsequent analyses were performed using MeV32,33. Gene Ontology (GO) analysis was performed using the DAVID Bioinformatics Resource34–36. For transcript quantitative analysis by qPCR, RNA was isolated as above and then converted to DNA using the Superscript III first-strand synthesis system (Invitrogen). Primer pairs and Roche Universal Probe information are listed in Supplementary Table 3. The comparative threshold method was used for relative quantification and each gene was normalized to GAPDH. Unless otherwise stated, a representative experiment is presented with error bars indicating s.e.m. of three technical replicates.

Cell culture, transient transfection, viral transduction, enzymatic assays. 293T and HT1080 cell lines (ATCC) were cultured in Advanced DMEM (Invitrogen) supplemented with penicillin-streptomycin (Invitrogen), glutamine (Invitrogen), and 10% newborn calf serum (Atlanta). Transient transfection, retroviral experiments: D.J.B., O.B. Analyzed the data: D.J.B., G.M., O.B., O.G. Wrote the main manuscript text: D.J.B., O.G. Reviewed the manuscript: D.J.B., G.M., O.B., O.G. The authors are grateful to members of the Gozani and Chua labs for insightful discussions regarding the work. D.J.B. was supported in part by a Stanford University DARE (Diversifying Academia, Recruiting Excellence) Doctoral Fellowship as well as a grant from the NIH (D01 GM079641). O.G. is a recipient of an Ellison Senior Scholar in Aging Award.

Author contributions

Conceived and designed the experiments: D.J.B., G.M., O.B. Performed the experiments: D.J.B., G.M., O.B. Analyzed the data: D.J.B., G.M., O.B. Wrote the main manuscript text: D.J.B., O.G. Reviewed the manuscript: D.J.B., G.M., O.B.

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Supplementary information

Table S1. Gene expression in support of the main text. 100% of patients with soft-tissue sarcoma were treated with Etoposide (100 μM) or vehicle (DMSO) for 1 hr, then prepared as described previously for chromatin immunoprecipitation (ChIP). Purified ChIP DNA was quantified by real-time PCR (RT-PCR) using a LightCycler 480 II (Roche) or a 7300 Real Time PCR machine (Applied Biosystems). Primers are listed in Supp Table 3. For direct ChIP experiments (unless otherwise indicated), a representative experiment is shown with error bars indicating standard error of the mean (s.e.m.) of three technical replicates. ChIP-chip assays were performed as previously described. Briefly, ChIP DNA purified as described above, then amplified (Sigma Whole Genome Amplification kits 2 and 3), and labeled, and hybridized to Roche Nimblegen 385 K ReSeq promoter arrays. ChIP-chip peaks were identified using the MACS algorithm as described in the manufacturer’s protocol. A peak must have a minimum of four consecutive ChIP-chip probes where the log2 ratio of ChIP to input signal is between 20-90% of the hypothetical maximum log2 ratio (the mean log2 ratio of the array + 6 standard deviations). The false discovery rate (FDR) for each peak was calculated by permutations of the data within each chromosome, our cutoff was FDR less than or equal to 0.2. We applied an additional cutoff which required the highest four probe peaks within the peak to have a log2 ratio > 0.5 (ING2 ChIP/input chromatin). To be a significant ING2 peak, the gene had to meet all of the criteria above in both biological replicates.

Antibodies and Plasmids. ING2 and derivative constructs were previously described. PIP4K beta and derivative constructs were previously described or subcloned into: pBabe with FLAG-HA epitopes. Mutants were made by PCR-mediated site-directed mutagenesis. Antibodies used in this study: ING2, H3K4me3 (Active Motif); SAP30, HDAC1 and Acetyl-H4 (Millipore); Rabbit IgG, FLAG M2 and H3Kac (Sigma).

Transcript analyses. RNA was isolated using RNeasy plus kits (Qiagen). For gene expression microarrays, RNA was processed for use with Illumina Bead Arrays per manufacturer’s instructions. Probe-level data were processed using Genespring software and all subsequent analyses were performed using MeV. Gene Ontology (GO) analysis was performed using the DAVID Bioinformatics Resource. For transcript quantitative analysis by qPCR, RNA was isolated as above and then converted to DNA using the Superscript III first-strand synthesis system (Invitrogen). Primer pairs and Roche Universal Probe information are listed in Supplementary Table 3. The comparative threshold method was used for relative quantification and each gene was normalized to GAPDH. Unless otherwise stated, a representative experiment is presented with error bars indicating s.e.m. of three technical replicates.

Cell culture, transient transfection, viral transduction, enzymatic assays. 293T and HT1080 cell lines (ATCC) were cultured in Advanced DMEM (Invitrogen) supplemented with penicillin-streptomycin (Invitrogen), glutamine (Invitrogen), and 10% newborn calf serum (Atlanta). Transient transfection, retroviral experiments: D.J.B., O.B. Analyzed the data: D.J.B., G.M., O.B., O.G. Wrote the main manuscript text: D.J.B., O.G. Reviewed the manuscript: D.J.B., G.M., O.B.

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Author contributions

Conceived and designed the experiments: D.J.B., G.M., O.B. Performed the experiments: D.J.B., G.M., O.B. Analyzed the data: D.J.B., G.M., O.B. Wrote the main manuscript text: D.J.B., O.G. Reviewed the manuscript: D.J.B., G.M., O.B.
Additional information
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Supplementary information accompanies this paper at http://www.nature.com/scientificreports
Competing financial interests: Dr. Gozani is a co-founder of EpiCypher, Inc.

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