Targeting the CoREST complex with dual histone deacetylase and demethylase inhibitors

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Here we report corin, a synthetic hybrid agent derived from the class I HDAC inhibitor (entinostat) and an LSD1 inhibitor (tranylcypromine analog). Enzymologic analysis reveals that corin potently targets the CoREST complex and shows more sustained inhibition of CoREST complex HDAC activity compared with entinostat. Cell-based experiments demonstrate that corin exhibits a superior anti-proliferative profile against several melanoma lines and cutaneous squamous cell carcinoma lines compared to its parent monofunctional inhibitors but is less toxic to melanocytes and keratinocytes. CoREST knockdown, gene expression, and ChIP studies suggest that corin’s favorable pharmacologic effects may rely on an intact CoREST complex. Corin was also effective in slowing tumor growth in a melanoma mouse xenograft model. These studies highlight the promise of a new class of two-pronged hybrid agents that may show preferential targeting of particular epigenetic regulatory complexes and offer unique therapeutic opportunities.
Epigenetic regulation of gene expression by histone modification has emerged as a major facet of physiologic and disease processes. As a result, there has been intense interest in developing epigenetic therapies leading to the discovery of small molecule agents that target proteins involved in histone modification. Several histone deacetylase (HDAC) inhibitors like vorinastat and panobinostat are now approved drugs for a specialized group of hematologic malignancies but not yet for a wider range of cancer types including solid tumors. One of the conceptual challenges in targeting HDACs is that even selective class I HDAC inhibitors such as entinostat (MS-275) likely impact these deacetylase activities indiscriminately across a range of distinct HDAC-containing multiprotein complexes. Such broad cellular effects may result in a narrow therapeutic window between disease efficacy and toxicity. Among HDAC complexes, the CoREST complex, which includes HDAC1 or its close paralog HDAC2, the scaffolding protein CoREST, and lysine specific demethylase 1 (LSD1) has attracted special interest. The HDAC1/2 and LSD1 enzymatic activities within the CoREST complex are commonly associated with silencing gene expression and contribute to cancer and other diseases. Several classes of LSD1 demethylase inhibitors have been reported, and the best characterized are analogs of tranylcypromine and phenelzine, established monoamine oxidase (MAO) mechanism-based inactivators (Supplementary Fig. 1). These MAO inhibitor analogs can be oxidized by LSD1’s active site flavin cofactor (FAD) and converted to reactive electrophiles that undergo covalent bond formation with FAD resulting in an irreversible blockade of LSD1. These tranylcypromine and phenelzine analogs have been shown to have anti-tumor potential alone and in combination with HDAC inhibitors in preclinical settings.

We considered the possibility that dual action LSD1/HDAC inhibitory compounds might be pharmaceutically advantageous. By comprehensively blocking the CoREST complex, dual LSD1/HDAC inhibitors could show a uniquely favorable profile of pharmacologic action with an improved therapeutic window relative to pure HDAC inhibitors. However, the challenges of developing pharmacologically attractive dual action CoREST inhibitors include: retaining high potency and specificity within

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**Fig. 1** Strategy for combining the pharmacophores of clinically used HDAC inhibitors and a preclinical LSD1 inhibitor to generate dual action HDAC-LSD1 inhibitors. Blue—incorporated LSD1 inhibitor features, Green—incorporated HDAC inhibitor features, Orange—shared structural features, Black—features not incorporated into dual inhibitors.
one compound for the two enzyme targets, achieving an approximate balance in targeting LSD1 and HDAC1/2 in the CoREST complex, and managing the size and polarity of such hybrid compounds to have a favorable pharmacologic profile.

**Results**

**Design and enzymatic analysis of dual LSD1/HDAC inhibitors.** Interestingly, compound 4SC-202 is proposed to act as a dual LSD1/HDAC inhibitor. In our assays, we did not observe significant inhibition of LSD1 with 4SC-202 (Supplementary Fig. 2) so it would appear to have a different mechanism of action from the inhibitors we report here (see below).

As a three-dimensional structure of the core CoREST complex was not available to aid our design, we based our strategy on current knowledge of pharmacophore characteristics of existing unifunctional HDAC and LSD1 active site targeting compounds. Inspection of several known HDAC and LSD1 inhibitor scaffolds suggests that a common phenyl ring could serve as the central element of an effective dual LSD1/HDAC inhibitor as shown (Fig. 1). Thus, we designed and synthesized hybrid compounds 1–6 that contained standard HDAC zinc binding groups, a benzamide (as in MS-275/entinostat) or a hydroxamic acid (as in SAHA/vorinostat and LBH589/panobinostat), tethered to an amine oxidase warhead, either phenelzine or tranylcypromine (Fig. 1). Details of the synthetic routes, related to prior efforts on related molecules, are shown in the Supplementary Methods. Compound 7 was new to this study, but is the cyclopropylamine analog of the established LSD1 inhibitor bizine. We also synthesized compounds 8 and 9 as controls that were closely related to 6 and 2 respectively, but included subtle warhead disruptions. Note that each of the designed bifunctional compounds 1–6 have molecular weights (< 500 g mol⁻¹) and hydrophobicity (0.5 < cLogP < 3) that are in the drug-like range (Supplementary Table 1).

We proceeded to determine the potencies of each of the designed hybrid compounds 1–6 against LSD1, as well as the enzymatically related off-targets HDAC2, and MAOs A and B. These hybrid compounds 1–6 were quite potent LSD1 histone demethylase inhibitors (Table 1), comparable to the parent phenelzine and tranylcypromine analogs from which they were derived. Each displayed time-dependent LSD1 inhibition suggesting mechanism-based inactivation with $k_{\text{inact}}/K_{i(\text{inact})}$ values that were within 3-fold of the parent mono-targeted LSD1 blockers. These compounds also showed moderate to strong selectivity against MAO A, MAO B, and LSD2 (Table 1, Supplementary Table 2, Supplementary Figs. 3–13). Hybrid compounds 1–6 were also tested against HDAC1 and the hybrid analogs 1–4 and 6 showed submicromolar potency whereas analog 5 was weaker, consistent with known structure-activity relationships related to linker length (Table 1, Supplementary Fig. 14). Pan-HDAC assays (HDACs 1–9) revealed that the benzamide compounds 1 and 2, analogs of class I HDAC inhibitor MS-275, were >100-fold selective for HDACs 1–3 as desired (Supplementary Fig. 15).

**Corin shows sustained inhibition of the core CoREST complex.** As CoREST complexes contain HDAC1 or 2, and compound 2 lacks the metabolic liability of the hydrazine substituent found in compound 1, we further examined the inhibitory properties of the selective dual inhibitor 2, hereafter called corin, with the purified core CoREST complex, prepared recombinantly from a mammalian cell expression system (Table 2). Size exclusion chromatography revealed stable association of the ternary protein complex and demonstrated 1:1: HDAC1:LSL1:CoREST stoichiometry (Fig. 2a, Supplementary Fig. 16). Kinetic analysis of the reconstituted CoREST complex HDAC1 component showed that activity was linear with time and has $k_{\text{cat}}/K_{m}$ values about 4-fold greater than commercial isolated HDAC1 using the well-established fluorescent peptide HDAC assay (Supplementary Fig. 17a–c). For reasons that are not yet clear, the LSD1 activity was observed to be bi-phasic, with an initial linear phase of 3–5 min (Supplementary Fig. 17d), and we consequently focused on this first phase for inhibitor analysis. Under our experimental conditions, corin and MS-275 were similarly potent in blocking CoREST complex HDAC1 activity, and corin matched the potency of the tranylcypromine analog 7 and the clinical candidate GSK2879552 toward CoREST LSD1 demethylase activity (Fig. 2b, c). Furthermore, corin was able to inhibit the deacetylation of semisynthetic, reconstituted nucleosomes by the CoREST ternary complex (Fig. 2d).

To address the possibility that corin, because of its dual warheads, might show sustained HDAC inhibition of the CoREST complex compared to MS-275, we compared the HDAC inhibitory properties of corin to that of MS-275 after prolonged exposure.

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**Table 1 Summary of inhibitor properties toward isolated LSD1, isolated HDAC1, and MAO A**

| Cmpd ID | isol. LSD1 | MAO A | isol. HDAC1 |
|---------|------------|-------|-------------|
|         | $k_{\text{inact}}$ (min⁻¹) | $K_{i(\text{inact})}$ (µM) | $k_{\text{cat}}/K_{i(\text{inact})}$ (min⁻¹·µM⁻¹) | $k_{\text{cat}}/K_{i(\text{inact})}$ (min⁻¹·µM⁻¹) | IC₅₀ (µM) |
| 1       | 0.28 ± 0.05 | 0.51 ± 0.16 | 0.55 | 0.055 ± 0.001 | 0.158 ± 0.003 |
| 2 (corin)| 0.17 ± 0.03 | 0.10 ± 0.06 | 1.70 | 0.074 | 0.147 ± 0.007 |
| 3       | 0.20 ± 0.04 | 0.19 ± 0.06 | 1.05 | -0.06 | 0.156 ± 0.013 |
| 4       | 0.25 ± 0.06 | 0.39 ± 0.19 | 0.64 | 0.063 ± 0.003 | 0.090 ± 0.002 |
| 5       | 0.25 ± 0.07 | 0.27 ± 0.18 | 0.93 | 0.062 ± 0.004 | 1.34 ± 0.36 |
| 6       | ND         | ND     | -5   | 0.22 | 0.099 ± 0.003 |
| bizine  | 0.20 ± 0.05 | 0.15 ± 0.09 | 1.33 | 0.11 ± 0.11 | > 30 |
| GSK2879552 | 0.28 ± 0.05 | 0.37 ± 0.19 | 0.76 | ND | 0.37 |
| 7       | 0.17 ± 0.01 | 0.30 ± 0.02 | 1.70 | ND | > 30 |
| 8       | ND         | ND     | -5   | 0.24 | > 30 |
| 9       | ND > 20    | ND     | ND   | 0.254 ± 0.014 | 0.254 ± 0.014 |
| MS-275  | ND > 20    | ND     | ND   | 0.233 ± 0.026 | 0.233 ± 0.026 |
| LBH589  | ND > 20    | ND     | ND   | -0.003 | 0.040 ± 0.003 |
| SAHA    | ND > 20    | ND     | ND   | 0.140 ± 0.013 | 0.140 ± 0.013 |

LSD1 = 110 nM, substrate = 300 µM dimethyl histone H3K4me2; peptide; HDAC1 = 2.86 nM, substrate = 20 µM acetylated P53379-382 tetrapeptide RHKK(Ac); MAO A = 200 nM, substrate = 200 µM tyramine

*MAO A inhibition data for bizine was reproduced from ref. 7

±Dash marks indicate that compound was not evaluated in the corresponding assay. Data (mean ± SEM) are representative of at least two independent experiments.
We observed that 6 h after a 30 min exposure to corin, the HDAC1 activity of the CoREST complex was markedly reduced. In contrast, treatment of the CoREST complex with MS-275 or MS-275 plus bizine led to little loss in HDAC1 activity. We also carried out a parallel dialysis experiment after treatment with corin and MS-275 of the HDAC1-containing MiDAC complex (MIDEAS/DNTTIP1/HDAC1) that lacks an LSD1 subunit (Fig. 3b). Both MS-275 and corin lacked inhibitory effects after dialysis. These results suggest that corin’s sustained inhibition of CoREST complex HDAC activity may be related to its LSD1 interaction. To further investigate the mechanism of inhibition of the CoREST complex by corin, we performed a jump dilution experiment (Fig. 3c). In this assay, after a 30 min exposure of 0.5 µM CoREST complex to 5 µM compound (corin, MS-275, LSD1 inhibitor 7, or desamino-corin 9), the mixtures were diluted 200-fold and aliquots were removed at time points from 0 to 120 min.
for measurement of HDAC1 activity. The jump dilution assays confirmed that corin showed near irreversible inhibition of HDAC1 activity whereas the HDAC1 activity with MS-275, compound 7, compound 9, or a mixture of 7 and MS-275 was restored within 40% of the vehicle rate by the end of the 2h period. The different inhibitory kinetics between compound 9 and corin are particularly notable since their chemical structures are identical except for compound 9’s deletion of a nitrogen atom which enables its LSD1 warhead.

These results are consistent with a mechanism of dual engagement of the CoREST complex HDAC1 and LSD1 active sites by corin’s warheads that can confer long-lasting enzyme inhibition (Fig. 3d). The initial phase of inhibition in these kinetic studies (Table 2) do not support enhanced potency of corin over the mono-functional inhibitors, so it is unlikely that the active sites of LSD1 and HDAC1 in the CoREST complex support dual occupancy by corin in the baseline state. Interactions of one enzyme active site with one warhead of corin may lead to a high effective concentration of the second warhead for the other enzyme subunit which over time induces conformational changes in the protein complex facilitating concurrent engagement. Computational modeling of the subunit proteins suggests the plausibility of energetically stable conformations with surface loop changes that allow for dual occupancy of LSD1 and HDAC1 by corin, but future studies will be needed to understand the precise structural mechanisms behind corin’s inhibition of the CoREST complex.

### Table 2 Biochemical characterization of dual inhibitors against LSD1 and HDAC1 as part of the CoREST ternary complex

| Cmpd ID | CoREST complex (LSD1) IC₅₀ (µM) | CoREST complex (HDAC1) IC₅₀ (µM) |
|---------|-------------------------------|-------------------------------|
| 1       | 1.8 ± 0.3                     | 0.230 ± 0.014                 |
| 2 (corin) bicine | 0.33 ± 0.05                     | 0.206 ± 0.035                 |
| 7       | 1.4 ± 0.6                     | ND                            |
| 9       | 0.38 ± 0.01                   | ND                            |
| 10      | 0.36 ± 0.01                   | ND                            |
| 11      | 0.20                         | 0.328 ± 0.026                 |

(LSD1 assay: CoREST complex = 100 nM, substrate = dimethyl histone H3K4<sub>1,9</sub> peptide; HDAC1 assay: CoREST complex = 2 nM, substrate = 50 µM acetylated P53<sub>379-382</sub> tetrapeptide RNKK(Ac))

*Dash marks indicate that compound was not evaluated in the corresponding assay. Data (mean ± SEM) are representative of at least two independent experiments.

Notably, corin showed more powerful anti-proliferative action against WM983B cells relative to MS-275 with an IC₅₀ of ~200 nM, about 12-fold lower than that of MS-275 (Fig. 4c) and Supplementary Fig. 20a, e). In fact, corin (1 µM) consistently showed a greater anti-proliferative effect than MS-275 (1 µM) across a panel of 10 melanoma cell lines (Fig. 4c). We also observed that combining the monofunctional LSD1 inhibitor 7 (1 µM) along with MS-275 (1 µM) across these cell lines did not match the anti-proliferative effect of corin (1 µM) suggesting that the integration of the two inhibitory warheads in corin is critical for its pharmacologic action. Consistent with this idea, desamino-corin compound 9 with its LSD1 warhead disrupted has a less potent IC₅₀ of 5 µM, similar to that of MS-275 (Supplementary Fig. 20b). Interestingly, corin (1 µM) was non-toxic to primary human melanocytes in contrast to MS-275 (1 µM) (Fig. 4c).

To explore the possible role of the CoREST complex in mediating the more powerful anti-proliferative action of corin vs. MS-275 against WM983B cells, we used shRNA to stably knockdown CoREST1, confirmed by qRT-PCR and Western blot (Supplementary Fig. 20c, d). It was also observed that these CoREST1 knockdown WM983B cells grew at ~40% more slow rate relative to control WM983B cells, indicating the functional importance of CoREST1 in WM983B cell proliferation (Fig. 4d). We next performed a dose-response analysis of MS-275 and corin in the parental vs. the CoREST1 knockdown WM983B cells. Strikingly, knockdown of CoREST1 conferred a heightened sensitivity to the anti-proliferative action of MS-275 in WM983B cells, rendering a similar dose-response profile to that of corin, whose antiproliferative effects were not altered by CoREST1 knockdown (Fig. 4e, f, Supplementary Fig. 20e, f). In contrast, knockdown in WM983B cells of the distinct corepressor protein SIN3A which participates in an HDAC1 complex that does not include LSD1<sup>5</sup> does not sensitize cells to MS-275 and the selectivity margin between corin and MS-275 is maintained under these conditions (Fig. 4g, h, Supplementary Fig. 21a).

We also investigated the comparative pharmacology of MS-275 and corin in HCT116 colon cancer cells and the isogenic LSD1<sup>−/−</sup>-HCT116 cell line<sup>27</sup>. As observed previously, LSD1<sup>−/−</sup>-HCT116 cells show a diminished proliferation rate relative to the parental HCT116 cells (Fig. 5a, Supplementary Fig. 21b). Loss of LSD1 enhanced the sensitivity of HCT116 cells to MS-275, but not corin (Fig. 5b, c). Taken together with the CoREST1 and SIN3A knockdown experiments, these results suggest that the more powerful anti-proliferative action of corin vs. MS-275 in the melanoma and colon cancer cells analyzed may involve its more comprehensive targeting and sustained inhibition of the LSD1-containing CoREST complex relative to other complexes. Nevertheless, even with CoREST1 knockdown, corin retains potency suggesting it can also block class I HDACs in other contexts.

To further assess the mechanistic basis of corin vs. MS-275 in WM983B cells, we analyzed cellular gene expression profiles after 24 h treatment with 2.5 µM of each compound. Approximately 1300 genes were selectively up-regulated at least 2-fold by corin compared with MS-275 under these conditions (Fig. 5d, Supplementary Fig. 22a). Gene ontology analysis suggested that genes important for differentiation, as well as modulators of cell motility were significantly up-regulated by corin compared to MS-275 (Supplementary Table 3). In addition, a large number of tumor suppressor genes were preferentially induced by corin, many of which have been previously observed to be epigenetically silenced in cancer (Fig. 5e, Supplementary Tables 4 and 5). We examined several of these tumor suppressors by qRT-PCR including p21, CHOP, SIK1, MXD1 and several others, each of which showed dramatic increases after corin treatment vs. MS-275 treatment, confirming the microarray data (Fig. 5f, g, Supplementary Fig. 22b–m). As in the anti-proliferative findings, anti-tumor properties of corin. Corin was subjected to an NCI60 screen<sup>21, 22</sup> which demonstrated preferential growth inhibition of melanoma cells vs. other cancer cell lines, consistent with the known epigenetic influences in the development and progression of melanoma (Supplementary Fig. 18).<sup>23, 24</sup> We subsequently tested corin’s effects on malignant melanoma WM983B histone modifications. Western blot and ELISA revealed that corin stimulated global H3K9 acetylation (H3K9Ac), as well as H3K4 mono- (H3K4Me1), di- (H3K4Me2), and tri-methylation (H3K4Me3) (Fig. 4a, b, Supplementary Fig. 19). In comparison to MS-275, corin appeared to more potently (corin EC₅₀ 95 nM vs. MS-275 EC₅₀ 420 nM) and efficaciously induce cellular H3K9 acetylation. Although MS-275 lacks direct LSD1 inhibitory properties, its ability and that of other HDAC inhibitors to influence global H3K4 methylation has been noted previously, presumably resulting from indirect impact on cellular methyltransferase or demethylase enzymes.<sup>25, 26</sup>
none of these genes showed enhancement by combining MS-275 with compound 7, highlighting the pharmacologic advantage of the hybrid molecule over the separate unifunctional compounds. We also found that the preferential induction by corin vs. MS-275 of CHOP and MXD1 was abolished in the CoREST knockdown melanoma cells (Supplementary Fig. 23a, b). Using ChIP-PCR analysis, we observed that the promoter regions of the CHOP and MXD1 genes showed high occupancy by both LSD1 and HDAC1 compared with control genes SYN1 and VAMP7 which were not selectively induced by corin (Fig. 5h, i, Supplementary...
Fig. 5 Analysis of gene knockout effects in HCT116 cells and gene expression changes in melanoma associated with corin treatment. a Knockout of LSD1 in HCT116 cells decreased the rate of cancer cell proliferation by ~40% \((n = 4)\). b, c Knockout of LSD1 enhanced the potency of MS-275 but did not affect the potency of corin in HCT116 cells as determined by \(^{3}\)H\]thymidine incorporation after 48 h treatment \((n = 4)\). d, e Venn diagrams depicting increases in global and tumor suppressor gene transcription (fold change \(\geq 2.0, p < 0.05\), respectively, upon 2.5 \(\mu\)M MS-275 or corin treatment \((n = 3)\). f, g qRT-PCR validation of gene expression changes identified by microarray. WM983B melanoma cells were treated with inhibitor (2.5 \(\mu\)M) for 24 h prior to RNA isolation and analysis \((n = 3)\). h ChiP-PCR localized CoREST complex components HDAC1 and LSD1 to the promoter region of CHOP \((n = 3)\). i ChiP-PCR localized HDAC1, but not LSD1, to the promoter region of SYN1 \((n = 3)\). Data (mean \(\pm\) SEM) are representative of at least three independent experiments (unpaired t test, \(*p < 0.05, **p < 0.001\)).

Figs. 22n, 23c, d, 24a, b). These data suggest that corin’s dual targeting of LSD1 and HDAC1 in the CoREST complex may contribute to its enhanced cellular pharmacology in melanoma.

The effects of LSD1 and HDAC inhibitors as well as corin were also investigated with the primary human cutaneous squamous cell carcinoma cell lines IC1 and MET1 (Table 3 and Supplementary Figs. 25–27). In contrast to the melanoma lines examined, we found that both of these cutaneous cancer lines were quite sensitive to the potent monofunctional LSD1 inhibitors compound 7, GSK2879552, and to a lesser extent tranylcypromine (Table 3, Supplementary Figs. 25 and 26) but not to the MAO inhibitor pargyline which is devoid of LSD1 inhibitor activity. We also found that the proliferation of IC1 and MET1 were more powerfully inhibited by corin than either the LSD1 inhibitors or the mono-functional HDAC inhibitors MS-275 and compound 9 (Table 3, Supplementary Figs. 25 and 26). In the case of IC1 and MET1, the anti-proliferative effects of the mono-functional LSD1 inhibitors compound 7 and GSK2879552...
were partially additive with MS-275 when used in combination. But the IC50 of corin was still 5-fold and 2-fold lower in IC1 and MET1, respectively, than the combination of compound 7 and MS-275. Moreover, corin proved far less toxic to primary human keratinocytes than MS-275, which were rather resistant to LSD1 inhibitors (Supplementary Fig. 28). These data highlight the potential therapeutic advantage of the hybrid agent in cancer cells that are particularly LSD1 inhibitor sensitive.

**Pharmacologic analysis of corin using a mouse xenograft.** We next demonstrated that corin showed high metabolic stability in human plasma, human liver microsomes, and mouse liver microsomes (Supplementary Table 6), and was well-tolerated in mice for 10 days of once daily intraperitoneal (IP) administration of up to 30 mg kg\(^{-1}\) based on stable similar weight relative to vehicle (Supplementary Fig. 29). We went on to examine the ability of corin to impact tumor growth in a melanoma mouse xenograft model using SK-MEL-5 cells. Melanoma xenografts of SK-MEL-5 cells are well established\(^{29}\) and, importantly, corin showed comparable efficacy (IC50 ~130 nM) in this cell line as compared to WM983B cells (Supplementary Fig. 30). These studies showed that corin treatment (30 mg kg\(^{-1}\), IP, every 24 h) led to a 61% reduction in tumor volume after 28 days relative to vehicle (Fig. 6a), with body weights and blood cell counts being similar between the treated and control animals at the end of the study (Supplementary Fig. 31). We attempted treatment of these xenograft mice with the same dose and schedule of MS-275 but this proved too toxic as 6 of 10 animals died with 1 week so this arm was discontinued. Examination of the tumor tissue recovered from mice treated with corin vs. vehicle showed that tumor cells from the corin-treated animals displayed elevated H3K9 methylation, H3K4 dimethylation (Fig. 6b), and increased expression of \(p21\), \(CHOP\), and \(MXD1\) (Fig. 6c), consistent with the cell culture experiments. Moreover, tumor cells from corin-treated animals showed a reduction in the proliferation biomarker Ki67 suggesting that the corin-treatment was blocking proliferation of these cells (Fig. 6d). These results substantiate corin’s promise for in vivo anti-cancer applications.

**Discussion**

It is generally accepted that in the context of cancer, single agent/single target therapeutics are poorly effective because of cancer pathway redundancies and the emergence of drug resistance\(^{30}\). Thus, many anti-neoplastic treatment protocols involve two or more drugs to achieve synergistic efficacy and prevent clonal resistance\(^{31, 32}\). An alternative strategy could involve the development of one drug that has engineered multifaceted pharmacodynamics. There have been several reports both in the context of small molecules, as well as protein biologics to exploit and/or rationally incorporate multi-target pharmacology in anti-tumor agents\(^{33, 34}\). Targeting two enzymes in the same complex that are thought to work concertedly to epigenetically inactivate gene expression offers an opportunity for synergistic pharmacology. While bisubstrate analog inhibitors that bind bivalently to a single enzyme that follows a ternary complex mechanism are commonplace\(^{36}\), dual action inhibitors aimed at two different enzymes in a protein complex are not well-established; however, these studies lend credence to the feasibility of this strategy. A somewhat related bivalent approach targeting LSD1 and HDAC was recently reported although the hybrid compound is designed to split into LSD1 and HDAC inhibitor components after LSD1 inactivation and the CoREST complex targeting capability has not yet been assessed\(^{37}\).

Our findings suggest that comprehensively targeting the CoREST complex can enhance residence time and this is facilitated by the hybrid functionality in corin vs. monofunctional HDAC inhibitors. However, further genomic studies will be needed to provide a full understanding of the relative targeting of the CoREST complex by corin chromatin wide in cells. Regardless, our results substantiate that such targeting can offer possible anti-tumor efficacy in malignancies that are especially sensitive to LSD1 inhibitors such as cutaneous squamous cell cancer, as well as those that are resistant to such agents, as seen in melanoma. Beyond the potential value of comprehensive epigenetic complex targeting and enhanced residence time\(^{38}\), dual action inhibitors promote a balance in concomitant enzyme blockade at the single cell level and overcome the substantial regulatory challenge of advancing two separate compounds concurrently into clinical trials.
**Methods**

**LSD1 expression and inhibition assay.** LSD1 inhibition was determined using a previously developed horseradish peroxidase (HRP) coupled assay. Briefly, LSD1 (residues 171–852) containing a N-terminal GST-tag was overexpressed in E. coli and purified via affinity chromatography. GST-LSD1 concentration was determined by gel electrophoresis (~0.9 mg mL\(^{-1}\), ~8.8 μM) and aliquots were stored at ~80°C (storage buffer: 280 mM NaCl, 5.4 mM KCl, 20 mM NaHPO\(_4\), 3.6 mM MgCl\(_2\), 10% Glycerol, pH 7.0). The recombinant LSD1 was expressed in Escherichia coli (DE3) and purified with affinity chromatography by incubating the lysate with 1 ml of Anti-GST beads (GE Healthcare). The resin was collected and washed with wash buffer (50 mM Tris–HCl, pH 7.5, 50 mM KCl, 5% glycerol, 0.5% Triton X-100, pH 7.5 in the presence of 1× Roche Complete EDTA-free protease inhibitor cocktail). The cells were lysed by sonication (15 s on, 30 s off, 42% amplitude, 3 cycles) and insoluble fractions were pelleted by centrifugation at 2800xg for 10 min and resuspended in cold lysis buffer (50 mM Tris–HCl, pH 7.5, 50 mM KCl, 5% glycerol, 0.4% Triton X-100, 1 µg Roche EDTA-free Complete Protease Inhibitor cocktail). The peptide contains a fluorogenic reporter and an extinction coefficient of 26,000 M\(^{-1}\) cm\(^{-1}\) for the generated chromophore. Progress curves representing the concentration of product formed over time were plotted and subjected to a series of mathematical transformations to determine the kinetic parameters (\(k_{\text{cat}}\), \(K_{\text{m}}\)) for each compound. Inhibitor stocks were prepared in fresh aliquots were thawed and diluted with storage buffer (not containing BME) to a final concentration of 0.9 mg mL\(^{-1}\) (MIDEAS, DNTTIP1, HDAC1) was expressed in HEK293F cells which were lysed for slow, tight-binding inhibitors, all components of the reaction except for the inhibitor were mixed in the 96-well plate and incubated at room temperature for 10 min. The reaction was initiated by the addition of substrate and fluorescence was monitored as previously described. Data are representative of at least three independent experiments.

**HDAC1 inhibition assay.** HDAC1 inhibitory activity was determined for each compound using the Fluorogenic HDAC1 assay kit available from BPS Bioscience (50031). Minor deviations from the manufacturer’s instructions were employed and are described below. HDAC substrate 1 (50032) was used instead of DMSO substrate 3 (50037). HDAC substrate 1 consists of a tetrapeptide based on residues 379–382 (RHKK(Ac)) of the tumor suppressor protein P53, a known HDAC substrate. The peptide contains a fluorogenic reporter that is activated after deacetylation by the addition of Developer (50030). Reactions were carried out in 384-well plate format in 3% DMSO/water by 2-fold serial dilution (final concentration of DMSO in each reaction was 2%).

**Protein complex HDAC inhibition assay.** Inhibition of CoREST complex LSD1 activity was determined using the same assay kit described in the HDAC1 inhibition assay with minor differences. Reactions were carried out for 10 min using 50 μM HDAC substrate 1 and a final concentration of 2 nM CoREST ternary complex or 8 nM MiDAC ternary complex. Slow, tight-binding inhibitors were also accounted for as described for the HDAC1 inhibition assay. Data are representative of at least three independent experiments.

**CoREST complex (LSD1) inhibition assay.** Inhibition of CoREST complex LSD1 activity was determined using the same coupled assay described for isolated LSD1 with the following modifications. The CoREST ternary complex (120 nM) was incubated with varying inhibitor concentrations in the presence of 60 nM HEPE (pH 7.4), 0.12 mg mL\(^{-1}\) BSA, and 0.12 mM isonitrosyl hexahistopamine for 5 min at 25°C as a 50 μl reaction. After a 5 min preincubation, the coupling reagents 4-AP, DHBS, and HRP were added and the reaction was initiated by the addition of substrate. The final reaction volume was 60 μl and comprises 50 mM HEPEs (pH 7.4), 0.1 mg mL\(^{-1}\) BSA, 0.1 mM isonitrosyl hexahistopamine, 0.1 mM 4-AP, 1 mM DHBS, 60 μM diMeK4H31, 12 substrate, 0.04 mg mL\(^{-1}\) (906 mM) HRP, 100 nM CoREST ternary complex, and varying concentrations of inhibitor. Inhibitory activity was determined by examining changes in absorbance in 5 min. Reported kinetic parameters represent at least two independent experiments.

**MiDAC ternary complex production and purification.** The MiDAC complex (MIDEAS, DNTTIP1, HDAC1) was expressed in HEK293F cells which were lysed and dialyzed against 1 L of buffer (25 mM Tris–HCl, 50 mM KCl, 5% glycerol, 0.5% BSA, 0.1 mM inositol hexakisphosphate, 0.1 mM 4-AP, 1 mM TCEP, pH 7.5, 50 mM KCl, 5% glycerol, 0.5% BSA, 20 µM EDTA-free Complete Protease Inhibitor cocktail). The peptide contains a fluorogenic reporter and an extinction coefficient of 38,200 M\(^{-1}\) cm\(^{-1}\) immediately before use. 60 μl reactions containing 50 mM HEPEs (pH 7.5), 0.1 mM 4-aminoantipyrine (4-AP), 1 mM 3,5-dichloro-6-hydroxybenzenesulfonate (DHBS), 300 mM diMeK4H31, substrate (ARTK Me\(_2\)QTARKSTGGKAPRKQLA, K\(_c\) = 25 μM, K\(_\text{m}^{\text{cat}}\) = 3 min\(^{-1}\), 0.04 mg mL\(^{-1}\) (906 mM) HRP) (Worthington Biochemical Corporation, Lakewood, NJ) was incubated with varying concentrations of inhibitor were initiated by the addition of LSD1 (final [LSD1] = 110 nM). Changes in absorbance at 515 nm were monitored over 20 min at 25°C using a Beckman Instruments DU series 600 spectrophotometer. Product formation was calculated using the Beer’s law and an extinction coefficient of 26,000 M\(^{-1}\) cm\(^{-1}\) for the generated chromophore. Product formation was plotted and subjected to a series of mathematical transformations to determine the kinetic parameters (\(k_{\text{cat}}\), \(K_{\text{m}}\)) for each compound. Inhibitor stocks were prepared in 8% DMSO/water by 2-fold serial dilution (final concentration of DMSO in each reaction was 2%).

**CoREST complex production and purification.** Constructs of LSD1, HDAC1, and FLAG-tagged CoREST1 were each cloned into a pcDNA 3.0-based vector and concentrated. Complex concentration was determined via MicroBCA assay (ThermoScientific) with BSA as the standard. The final complex was then stored at 4°C. The complex was expressed using the same coupled assay described for isolated LSD1 with the following modifications. The CoREST ternary complex (HDAC) jump dilution assay. The CoREST ternary complex (0.5 μM buffer: 25 mM Tris–HCl, 50 mM KCl, 0.5 mM TCEP, pH 7.5) was incubated with the indicated inhibitor (5 μM, 2% DMSO, 100 μM isonitrosyl hexahistopamine) at 25°C for 30 min prior to drop dilution. The complex was then diluted to a final concentration of 2 nM in HDAC assay buffer (BPS Bioscience, 50031) containing 200 μM HDAC substrate 1 (BPS Bioscience, 50032) and 0.1 mg mL\(^{-1}\) BSA. 50 μl aliquots were quenched at 0, 5, 10, 20, 40, 120 min with an equal volume of Developer (BPS Bioscience, 50030) and fluorescence was measured as described in the HDAC1 inhibition assay. The complex was represented by 3-fold serial dilution. Data are representative of at least three independent experiments.

**CoREST complex (HDAC) jump dilution assay.** The CoREST ternary complex (0.5 μM buffer: 25 mM Tris–HCl, 50 mM KCl, 0.5 mM TCEP, pH 7.5) was incubated with the indicated inhibitor (5 μM, 2% DMSO, 100 μM isonitrosyl hexahistopamine) at 25°C for 30 min prior to drop dilution. The complex was then diluted to a final concentration of 2 nM in HDAC assay buffer (BPS Bioscience, 50031) containing 200 μM HDAC substrate 1 (BPS Bioscience, 50032) and 0.1 mg mL\(^{-1}\) BSA. 50 μl aliquots were quenched at 0, 5, 10, 20, 40, 120 min with an equal volume of Developer (BPS Bioscience, 50030) and fluorescence was measured as described in the HDAC1 inhibition assay. The complex was represented by 3-fold serial dilution. Data are representative of at least three independent experiments.

**Prolonged HDAC inhibition assay.** To determine if dual inhibitors could maintain inhibition of protein complex HDAC activity after extended dialysis, 0.5 μM CoREST or MiDAC ternary complex was treated with 5 μM inhibitor for 30 min at 4°C in 50 μl of 25 mM Tris–HCl, 50 mM KCl, 100 μM isonitrosyl hexahistopamine, and 0.5 mM TCEP, pH 7.5. After 30 min, the reactions were transferred to dialysis cassettes (Slide-A-Lyzer\(_{\text{TM}}\) 10 K MWCO Mini Dialysis Device, Thermo Fisher Scientific, Haltorf, MD) and dialyzed against 11 of buffer (25 mM Tris–HCl, 50 mM KCl, 0.5 mM TCEP, pH 7.4) three times for 2 h at each time at 4°C of note, it was necessary to keep the reaction temperature at the same level of the dialysis bag to avoid reaction dilution. After 6 h dialysis, the reactions were transferred to 0.5 mL Eppendorf tubes and the protein complex HDAC inhibition assay was carried out with the dialyzed complex. Inhibitor stocks were prepared, as described for the HDAC1 inhibition assay. Data are representative of at least two independent experiments.

**Nucleosome deacetylation assay.** N-terminal histone H3 tail peptides containing H3K9ac modifications were prepared using SPPS (0.1 mmol) and ligated to truncated, recombinant histone H3 via sortase ligation. Semisynthetic histone H3

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was refocused with other core histones followed by nucleosome reconstitution via gradient dialysis in the presence of nucleosomal DNA. 41 Kinase CoREST ternary complex (final CoREST:E1 = 1:100 M) was pre-treated with inhibitor at 25 °C for 30 min in 50 mM HEPES, pH 7.5, 100 mM KCl and 0.2 mg ml⁻¹ BS A. Reactions were initiated by the addition of nucleosome substrate (final [nucleosome] = 100 nM) with a final reaction volume of 50 μl. 10 μl aliquots taken at 0 h, 1 h, and 2 h were quenched with 10 μl EDTA and 4X sample loading buffer and analyzed by SDS-PAGE. Histone octamers were then refolded with other core histones to form histone octamers followed by analysis via Western blot. Nitrocellulose membranes were blocked with 5% BSA in TBST and incubated overnight with primary antibodies, either α-H3K9ac (Abcam, ab32129, 1:1000), α-H3K4me2 (Abcam, ab23356, 1:5000), α-H3K4me1 (Abcam, ab88959, 1:5000), α-H3K4me3 (Abcam, ab85880, 1:5000), α-H3K14ac (Millipore, 07-353, 1:5000), α-H3K18ac (Millipore, 07-364, 1:5000), α-DNAI (Abcam, ab17721, 1:5000), HDAC1 (Abcam, ab19868, 1:5000), total H3 (Cell Signaling Technology, 4499 S, 1:1000 or Abcam, ab1791, 1:1000), β-actin (Santa Cruz Biotechnology, sc7778, 1:2000), GoREST1 (BD Transduction Laboratories, 612146, 1:5000), SIN3A (Abcam, ab129087, 1:1000), HRP-conjugated secondary antibody (Santa Cruz Biotechnology, 1:5000 for β-actin blots, 1:2000 for all others). Blots shown are representative of at least two independent experiments. Uncropped versions of Western blots are provided in the Supporting Information (Supplementary Figs. 32 and 33).

**LSD2D1** was overexpressed in E. coli after which the cells were lysed using a French press with cold buffer (280 mM NaCl, 5.4 mM KCl, 20 mM NaHPO₄, 3.6 mM KH₂PO₄, 1.3 mM PMSF, 6.8 μg ml⁻¹ DNase I, 10% (v/v) glycerol, pH 7.4, 1:10 Roche Complete EDTA-free protease inhibitor). Lyase was clarified by centrifugation and the target protein purified by nickel affinity chromatography. 42 Inhibitor potency toward the LSD1 homolog, LSD2D1, was determined using a procedure similar to that described for LSD1 above 43, 44. Briefly, 60 μl reactions containing 50 mM HEPES, pH 7.5, 0.1 mM 4-AP, 1 mM DHBS, 100 μM diMeK4H3₂ac9₉, substrate, 0.04 mM mg⁻¹ (906 nM) HRP, and 20 μM inhibitor were initiated by the addition of LSD2D1 (final [LSD2D1] = 430 nM). Absorbance measurements and data processing were carried out as described for LSD1. Data are representative of at least two separate experiments.

**MAO A/B** counterscreen. MAO A (M7316), MAO B (M7441), and the tyramine substrate (T90344) were purchased from Sigma-Aldrich. MAO A (83.2 μM) and MAO B (83.5 μM) were aliquoted and stored at −80 °C until use according to the manufacturer’s instructions. MAO A was diluted 8-fold to 10.4 μM with 50 mM HEPES, pH 7.4 immediately before use. The tyramine substrate was stored at −80 °C as a 100 mM stock in DMSO and diluted to 0.8 mM (MAO A) and 0.5 mM (MAO B) with water immediately before use. In total 100 μl reactions containing 50 mM HEPES, pH 7.5, 0.1 mM 4-AP, 1 mM DHBS, tyramine substrate (1 μM), α-mylene (200 μM for MAO A (k₉ = 29 μM, k₉ = 93 min⁻¹) and 125 μM for MAO B (k₉ = 93 μM, k₉ = 0.20 min⁻¹) assays), 0.04 mg ml⁻¹ (906 nM) HRP, and varying concentrations of inhibitors were initiated by the addition of either MAO A or B (final [MAO A] = 0.2 μM, final [MAO B] = 1.67 μM) 45. Inhibitor stocks were prepared as before and absorbance measurements and data processing were carried out as described for LSD1. Data represent realized at least two separate experiments.

**HThymidine incorporation assays.** Cells were seeded in a 96-well plate and treated with inhibitor at the indicated concentrations. Media was removed and replaced with fresh media containing compound every 24 h. After 7 h incubation, 20 μl lysis buffer (10 mM Tris-HCl, 1 mM EDTA, 0.2% (v/v) Triton X-100) was added to each well and the plate was shaken vigorously on an orbital shaker for 10 min. 70 μl TE buffer was subsequently added to each well and the plate was agitated for 5 min. Then, 95 μl of sample solution from each well was transferred to a new 96-well, non-clear bottom plate (NUC3 236105 96 F) and to each well was added 99 μl of 1X Picogreen® solution (Life Technologies, P11496). The plate was then agitated for 5 min in the dark after which fluorescence was measured using excitation and emission wavelengths of 480 nm and 520 nm, respectively, using a SpectraMax microplate reader. Data represent at least two independent experiments with three technical replicates per experiment. Where P-values are reported, the unpaired t test was used to determine significance.

**CoREST and SIN3A knockdown.** shRNA clones targeting CoREST (TRCN0000128570 and TRCN0000129660) or SIN3A (TRCN000021774) were obtained from the Open Throughput Biology Center at Johns Hopkins University.

**Lentiviral particles were produced in HEK293T cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions.** Cells were cultured in DMEM with 5% FBS and 80 μg ml⁻¹ polybrene at 37 °C after 0.22 μm filtration. For lentiviral infection, WM983B cells were incubated with CoREST shRNA, SIN3A shRNA, or scramble containing lentiviral particles overnight. Cells were selected with puromycin 48 h after transduction to create stable cell lines. CoREST knockdown was determined by quantitative RT–PCR and confirmed by Western blot. shRNA sequences are provided as Supplementary Table 7.

**Whole genome microarray.** WM983B melanoma cells were treated for 24 h with 2.5 μM corin, MS-275, or DMSO as a control. RNA was isolated from cells following the manufacturer’s instructions and cleaned using NucleoPure Mini kit (Qagen Inc.). The isolated RNA was hybridized to the Affymetrix Human Gene 2.0 ST arrays at the Johns Hopkins Microarray Core Laboratory, including quality control for extracted total RNA samples. Experiments were performed according to
the manufacturer’s recommended protocols. In brief, the total extracted RNA was converted into cDNA, amplified, purified, and then labeled. Labeled cDNA was hybridized to HuGene2.0 ST array and was scanned using Affymetrix GeneChip scanner 3000 with G7 upgrade (Affymetrix GeneChip Expression Analysis Technical Manual, Affymetrix, Santa Clara, CA). Data are representative of three independent experiments.

**Microarray data analysis.** We extracted raw gene expression data, Affymetrix exon CEL files, and RNA (Robust Multi-array Average)5,41,42 and normalized them using Partek Genomics Suite v6.6 analytic platform (Partek Inc., St. Louis, MO). We converted the replicate cell samples’ gene expression values to log2 notation and then compared the corresponding corin and MS-275 samples using a one-way ANOVA model. We visualized the ANOVA results of this comparison as volcano plots using the Spotfire DecisionSite platform (TIBCO Software Inc., Palo Alto, CA). We then performed further functional and pathway analyses to illustrate underlying biologic mechanisms. Since the log2 fold changes (log ratios) of the arrays’ 38,598 gene-annotated transcripts presented a normal distribution, their SD (standard deviation) from the mean of no change was set to the threshold for highly up- and down-regulated genes that were used in pathway analysis. The set of all ANOVA results was exported from Partek to the QIAGEN Ingenuity Pathway Analysis platform (Qagen Inc., Valencia, CA), where those genes showing greater than 2 SD change up or down were compared to the universe of all the array’s genes. This 2 SD value corresponds to a linear expression fold change of ~1.952 and comprised 1843 unique genes. Ingenuity software used the Fisher exact test to identify biological functions that were statistically enriched for 2 SD differentially expressed genes above what would be expected at random.

**Quantitative real time-PCR.** RNA was isolated from primary human melanocytes and melanoma cells following the manufacturer’s instructions and cleaned using the RNeasy Mini kit (Qiagen Inc.). In total 1 µg of RNA was reverse transcribed using SuperScript™ III Reverse Transcriptase and Random Hexamer primer kit (Invitrogen). PCR’s quantitative was performed for 40 cycles of 15 s at 95 °C and 30 s at 60 °C, using the Step One Plus Real-time PCR system (Applied Biosystems). Data represent two independent experiments with three technical replicates per experiment. The data was calculated using the delta (delta Ct) method. Where p-values are reported, the unpaired t test was used to determine significance. Transcripts were amplified using the primers listed in Supplementary Table 8.

**Chromatin immunoprecipitation assay (ChIP-PCR).** VM983B cells (10 × 10^6) were crosslinkered in 1% formaldehyde for 7 min and lysed in cell lysis buffer (5 mM HEPES, 85 mM KCl, 0.5% NP-40, 1× protease inhibitor cocktail (Sigma), 1× phosphatase inhibitor cocktail (Sigma)) on ice for 20 min. The nuclei were lysed in buffer containing 50 mM Tris–HCl pH 8, 10 mM EDTA, 1% SDS and then chromatin was fragmented using the BioRuptor (Diagenode) sonicator to obtain an average size of 250–300 base pairs. Chromatin concentration was measured by its absorbance at 260 nm and the Nanodrop (1 unit ml^-1 of chromatin corresponds to OD_{260} = 1) and diluted 1:10 with dilution buffer (165 mM NaCl, 0.01% SDS, 1.1% Triton X-100, 16.7 mM Tris–HCl, pH 8.0). 1 unit of chromatin was immunoprecipitated by protein A magnetic beads (Dynabeads, Invitrogen) after incubation with antibodies specific for HDAC1 (Abcam, ab19845, 1:1000) and LSD1 (Abcam, ab17721, 1:500). Normal goat IgG and non-antibody treated samples were used as negative controls. Following overnight immunoprecipitation, beads were washed twice consecutively with each of the following buffers: LiCl-B (50 mM HEPES, pH 8.0, 140 mM NaCl, 1% Triton X-100, 0.1% sodium deoxycholate, 1 mM EDTA), Hio-B (50 mM HEPES, pH 8.0, 500 mM NaCl, 1% Triton X-100, 0.1% sodium deoxycholate, 1 mM EDTA), LiCl (10 mM Tris–HCl, 250 mM LiCl, 0.5% NP–40, 0.5% sodium deoxycholate, 1 mM EDTA) and TE (10 mM Tris–HCl, 1 mM EDTA). Immunoprecipitated chromatin and input DNA were reverse crosslinkered in elution buffer (50 mM Tris–HCl, 10 mM EDTA, 1% SDS) in the presence of proteinase K (50 µg ml^-1) by shaking (1300 RPM) at 68 °C for 5 h. DNA was purified using phenol-chloroform and precipitated in ethanol at −20 °C. DNA pellets were dissolved in 200 µL of dH2O. The relevant primers are listed in Supplementary Table 8.

**Plasma stability studies.** Drug-free (blank) human plasma originated from Biologie Specialty Corp. (Colmar, PA, USA). Plasma studies were conducted in human plasma and 500 ng ml^-1 of corin in a final volume of 500 µL. Incubations were performed in duplicate in glass tubes maintained at 37 °C in a shaker bath. Stability studies were terminated immediately or after 0.5 or 1 h by taking 20 µl of the microsome mixture and adding 1 ml of acetonitrile, followed by vortex-mixing then centrifugation for 10 min at 1430×g. A 10 µl aliquot of the supernatant was injected onto the UPLC instrument for qualitative analysis using a temperature-controlled autosampling device operating at 5 °C.

**LCMS analysis.** Chromatographic analysis was performed using a Waters Acquity Ultra Performance UPLC. Separation of the analyte from potentially interfering material and metabolites was achieved at ambient temperature using Waters X-bridge column (50 × 2.1 mm, 1.7 µm) with a 2.7 µm particle size. (Milford, MA). The mobile phase used for the chromatographic separation was composed of 0.1% (v/v) formic acid in water (mobile phase A) and 0.1% (v/v) formic acid in methanol (mobile phase B) with a flow rate of 0.3 ml min^-1. The initial mobile phase composition was 90% mobile phase A and 10% mobile phase B. From 0 for 1 week prior to beginning the experiment. For each animal, SK-MEL-5 (5 × 10^6) cells in 150 µl growth media mixed with 50% matri-gel (BD, USA) were injected into the subcutaneous tissue of the flank^29,47. When xenograft size reached an approximate volume of 150 mm^3, the 20 mice were randomized into 10 groups with the average tumor volume distributed equally between groups (vehicle: avg. initial mouse body weight was 18.4 ± 0.3 g, avg. tumor volume was 150 ± 9 mm^3), corin: avg. initial mouse body weight was 18.3 ± 0.2 g, avg. initial tumor volume was 151 ± 7 mm^3) was used to evaluate MS-275 (30 mg kg^-1) head to head with corin. However, six of the ten animals died within one week of initiating the study leading to early termination of this arm for ethical reasons. The mice were maintained in a pathogen-free environment with free access to food and water. Body weight and tumor volume were measured twice a week. Tumor size was measured with linear calipers and calculated using the formula: (length in millimeters × width in millimeters)^2/2). The mice were sacrificed after 4 weeks and tumor weights were measured. Three animals from each treatment group were chosen at random for follow up blood work. Where p-values are reported, the unpaired t test was used to determine significance.

**Xenograft tumor sample processing and immunohistochemistry.** After sacrificing the mice, tumors were removed and post-fixed in 3.5% formaldehyde. Serial sections of the block were cut into the formalin fixed, paraffin embedded tissue blocks, floated onto charged glass slides (Super-Frost Plus, Fisher Scientific), and dried over night at 60 °C. Sections were deparaffinized and hydrated using graded concentrations of ethanol to deionized water prior to immunohistochemistry. Tumor sections were blocked in serum (5% serum in PBS-T (0.5% Triton-X100 in PBS)), and then incubated overnight at 4 °C in rabbit anti-Ki67 (Abcam, ab5580, 1:500 dilution) and then incubated in goat anti-rabbit secondary antibody for 1 h at room temperature, washed with PBS, and mounted using VECTASHIELD mounting medium (Vector Laboratories).
Supplementary Data 1. The additional data that support the findings of this study are available from the corresponding authors upon request.

Received: 9 October 2017 Accepted: 14 November 2017 Published online: 04 January 2018

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Acknowledgements

We thank the NIH, the FAMRI foundation, the V foundation, the Italian Association for Cancer Research (IG15208 and IG19162) for financial support. This work was supported by PBN 2016 (prot.20152TE5PK) (A.M.), NIH (R01GM114306) (A.M.), and the EMBO ALTF 261-2012 (V.A.G.) funds. J.W.R.S. is a Wellcome Trust Senior Investigator (grant WT100237) and Royal Society Wolfson Research Merit Award Holder. He is also supported by a Biotechnology and Biological Sciences Research Council Project Grant BB/J009598/1 as well as funds from 45C. A.D. K. is supported by a program award from Cancer Research UK (C20953/A18644), a Biotechnology and Biological Sciences Research Council Project Grant BB/L0123X/1, and a British Skin Foundation Project Grant 2015. A.E. was a Kagome Visiting Scholar. We thank Xiaowen Yang and Peter Watson for help with the protein expression and purification. We thank R. Casero for the HCT116 LSD1 knockout cells and D. Meyers, C. Talbot, and J.W. Labonte for helpful advice in regard to synaptic strategy, microarray data analysis, and computational modeling, respectively.

Author contributions

J.K., J.D., A.G., and X.V. designed and synthesized the dual inhibitors with contributions from P.P., A.M., and P.C. I.K., A.G., D.H., N.A., M.W., and J.R. carried out the biochemical characterization. Y.S. and J.S. established construct boundaries and protocols to express, purify, and characterize a stable CoREST ternary complex in mammalian cells. Y.S., D.H., and M.W. expressed and purified the CoREST ternary complex for enzyme
assays. L.F. and C.M. expressed and purified the MiDAC complex. J.J. and S.R.B. modeled the corin bound CoREST complex. M.W. performed analyses of compounds in melanoma cell lines. M.W. and H.J.C. performed analyses of immunostaining and molecular studies on melanoma xenograft tissues. M.W. performed gene silencing experiments for CoREST and related epigenetic modifying genes in melanoma cell lines and all associated analyses with the assistance of E.K. and H.R. M.W., J.K., and N.A. performed microarray studies and analyses with the assistance of B.R. M.W. performed ChIP studies and analyses with the assistance of E.K. and I.P. J.K, A.G., E.K., H.R, and A.E. performed additional cell-based work with design contributions from A.D.-K., W.H., B.R., A.R., and P.C. C.P. provided the SCC cell lines. N.M.A. carried out the drug stability studies with contributions from M.R. Y.H. and M.W. carried out mouse xenograft studies with the assistance of B.R. and I.P. Data analysis was supervised by A.D.-K., J.G., J.B., J.S., A.M., R. A., and P.C. J.K. and P.C. drafted the manuscript. All authors were involved in the experimental design, data interpretation, and manuscript editing.

Additional information
Supplementary Information accompanies this paper at https://doi.org/10.1038/s41467-017-02242-4.

Competing interests: The chemical material presented is the subject of provisional patent application WO 2015/134973 titled “A Selective Phenelzine Analog Inhibitor of Histone Demethylase LSD1”.

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Jay H. Kalin, Muzhou Wu, Andrea V. Gomez, Yun Song, Jayanta Das, Dawn Hayward, Nkosi Adejola, Mingxuan Wu, Izabela Panova, Hye Jin Chung, Edward Kim, Holly J. Roberts, Justin M. Roberts, Polina Prusevich, Jeliazko R. Jeliazkov, Shourya S. Roy Burman, Louise Fairall, Charles Milano, Abdulkerim Eroglu, Charlotte M. Proby, Albena T. Dinkova-Kostova, Wayne W. Hancock, Jeffrey J. Gray, James E. Bradner, Sergio Valente, Antonello Mai, Nicole M. Anders, Michelle A. Rudek, Yong Hu, Byungwoo Ryu, John W.R. Schwabe, Andrea Mattevi, Rhoda M. Alani & Philip A. Cole

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