Merkel cell polyomavirus activates LSD1-mediated blockade of non-canonical BAF to regulate transformation and tumorigenesis

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Merkel cell carcinoma (MCC)—a neuroendocrine cancer of the skin—is caused by the integration of Merkel cell polyomavirus and persistent expression of large T antigen and small T antigen. We report that small T antigen in complex with MYCL and EP400 (p400) transcriptional activator complex. This activity is required for the small T antigen-mediated transformation of normal Merkel cells, including expression of KRT20 (CK20) and chromogranin A.

MCV small T antigen recruits MYCL (L-Myc) and MAX to the EP400 (p400) transcriptional activator complex. This activity is required for the small T antigen-mediated transformation of normal cells. To identify genes regulated by the small T antigen–MYCL–EP400 complex, chromatin immunoprecipitation sequencing (ChIP-Seq) of small T antigen, MAX and EP400 and RNA sequencing (RNA-Seq) following depletion of EP400, MYCL or small T antigen was performed in MCC cell lines. One validated target gene of the small T antigen–MYCL–EP400 complex is MDM2, which represses wild-type p53 activity in virus-positive MCC.

Lysine-specific histone demethylase 1 (LSD1) is a histone demethylase that removes H3K4 mono- and dimethylation transcription marks. An alternatively spliced form of LSD1 (+8a) can demethylate H3K9me. LSD1 assembles into a core transcriptional repressor complex containing RCore1 (CoREST), RCore2 or RCore3 and is recruited to chromatin by the SNAG domain-containing proteins INSM1, GFI1, GFI1B, SNAI1 and SNAI2, which play key roles in development and oncogenesis.

Small-molecule inhibitors of LSD1 have promising activity in preclinical models of acute myeloid leukaemia, small cell lung cancer and medulloblastoma. In addition to inactivating its enzymatic activity, some LSD1 inhibitors disrupt the interaction between LSD1 and SNAG domain proteins and the interaction with chromatin. The exact mechanism of how LSD1 inhibition interferes with cancer cell growth is yet to be determined.

The mammalian SWI/SNF (mSWI/SNF) and BRG1/BRM-associated factor (BAF) complexes contribute to the regulation of genes involved in differentiation. Over 20% of all human cancers harbour mutations in mSWI/SNF complex components. The 29 gene products assemble combinatorially to produce three related mSWI/SNF complexes: canonical BAF, polybromo-associated BAF (PBAF) and non-canonical BAF (ncBAF). Each complex contains SMARCA4 (BRG1) or SMARCA2 (BRM) but they are distinguished by complex-specific subunits. BRD9—a BET family protein that reads acetylated lysine histone marks—is unique to the ncBAF complex along with GLTSCR1 (BICRA) and GLTSCR1-like (BICRAL). Targeting the ncBAF complex confers synthetic lethality in synovial sarcoma and malignant rhabdoid tumours, and mis-splicing of BRD9 provides growth advantages in SF3B1-mutated cancers, suggesting a specific role in cancer.

Results

MCV small T antigen activates the LSD1 repressor complex. RNA-Seq performed on MCC cell lines after RNA interference-mediated knockdown of MYCL, EP400 or small T antigen identified...
changes in the levels of many expressed genes. Integrative analysis of RNA-Seq and ChIP-Seq revealed that reduced levels of genes were significantly associated with promoters bound by small T antigen, MAX and EP400 (Extended Data Fig. 1a). In contrast with genes significantly associated with promoters bound by small T antigen, RNA-Seq and ChIP-Seq revealed that reduced levels of genes were affected by LSD1, RCOR2 and INSM1, RNA and protein levels were assessed in PHL cells with or without MCV T antigens (Fig. 1g,h). T antigens increased the levels of LSD1 and RCOR2 but not INSM1. Furthermore, LSD1 could specifically co-immunoprecipitate RCOR2 in PHL cells with MCV T antigens (Fig. 1i). ChIP-qPCR for small T antigen, MAX and EP400 was performed to assess enrichment for the promoters of LSD1 and RCOR2 (Fig. 1j). We found that MAX and EP400 enrichment increased in the presence of T antigens. These results indicate that small T antigen—MYCL—EP400 can activate expression of the LSD1 complex components. Of note, expression of a small T antigen mutant unable to bind the EP400 complex in PHL cells (PHL2) increased the levels of LSD1 and RCOR2, suggesting that T antigens may have additional means to activate these genes (Extended Data Fig. 1f,g).

**LSD1 inhibition abrogates T antigen-driven transformation.**

We assessed RNA and protein levels of RCOR2, LSD1 and INSM1 in several virus-positive (MKL-1, MKL-2, MS-1, WaGa, PeTa and BroLi) and virus-negative MCC cell lines (UISO, MCC13 and MCC26) and human foreskin fibroblasts (HFFs). LSD1, RCOR2 and INSM1 transcripts (Fig. 2a–c) and protein (Fig. 2d) were significantly more abundant in the virus-positive MCC cell lines than in virus-negative MCC cell lines or HFFs. Although there is controversy regarding the ontology of the virus-negative MCC cell lines, the results showed that MCC cell lines expressing T antigens expressed LSD1, RCOR2 and INSM1 abundantly.

To determine whether MCC cell lines were sensitive to LSD1 inhibition, we treated the same panel of MCC cell lines with the LSD1 inhibitors GSK2879552, CPI-242 and GSK-LSD1 (ref. 11). Cell viability was assessed by CellTiter-Glo assay after 12 h of treatment (Fig. 2f and Extended Data Fig. 2). All virus-positive MCC cell lines tested were sensitive to nanomolar concentrations of LSD1 inhibitors, whereas the virus-negative cell lines were highly resistant.

To determine whether the transformation of normal cells by MCV T antigens required LSD1 activity, we performed an anchorage-independent growth assay using MKL-1 and PHL cells with MCV T antigens (Fig. 2g,h). Addition of the GSK-LSD1 inhibitor reduced the transformed phenotype of PHL cells with MCV T antigens, as shown by the reduced number of colonies formed when cultured in soft agar. Similarly, virus-positive MKL-1 MCC cells were able to grow in soft agar, but treatment with the GSK-LSD1 inhibitor completely blocked colony formation.

**Integrated LSD1 target analysis reveals critical gene expression changes during LSD1 inhibition.**

The virus-positive MCC cells grew as loose clusters in suspension and formed tight floating spheroids in the presence of LSD1 inhibitors (Fig. 2i). To assess for changes in gene expression, RNA-Seq was performed on six virus-positive cell lines and the virus-negative UISO MCC cell line treated with various LSD1 inhibitors for 1 or 3 days (Fig. 3a–c). Despite differences in the treatment parameters, similar gene expression changes were observed in response to LSD1 inhibition in all six virus-positive cell lines but not in the UISO cells when assessed by RNA-Seq and RT-qPCR (Fig. 3b,d). Principal component analysis (PCA) of the RNA-Seq data showed similar global shift changes in gene expression in all six virus-positive MCC cell lines with LSD1 inhibition (Fig. 3c). The levels of many genes, including FAM5B, CDH11, CALB2, PROM1, GFI1, SMARCA1, SMAD9, ID2 and HEY2, were significantly increased by LSD1 inhibition in virus-positive cells but not in virus-negative cells (Fig. 3a,b,d and Extended Data Fig. 3a,b,d). These changes were accompanied by increased levels of H3K4me1 but not H3K4me2, suggesting that LSD1 inhibition specifically targets mono-methylation of H3K4 in MCC cells (Fig. 3c).

The LSD1 target gene analysis predicted that bone morphogenetic protein (BMP) signalling factors, including inhibitors of DNA binding 1 (ID1), ID2, ID3 and SMAD9 were targets (Fig. 3 and Extended Data Fig. 3b–d). The BMP pathway regulates embryonic patterning and neuroectodermal development. Soluble BMP proteins bind to their receptors to promote phosphorylation of SMAD1, SMAD5 and SMAD9, which, in turn, transactivates specific target genes, including inhibitors of DNA binding22. To determine whether LSD1 inhibition activates BMP, we performed western blotting and observed increased phosphorylation of SMAD1, SMAD5 and SMAD9, as well as increased levels of ID1 RNA and protein (Fig. 3e–h and Extended Data Fig. 3c). To test the specificity of the LSD1 inhibitors, we depleted LSD1 using a short hairpin RNA (shRNA) in MKL-1 and WaGa cells and assessed the levels of LSD1 target genes by western blotting and observed increased phosphorylation of SMAD1, SMAD5 and SMAD9, as well as increased levels of ID1 RNA and protein (Fig. 3e–h and Extended Data Fig. 3c).

To identify genes directly regulated by the LSD1 repressor complex, we performed ChIP-Seq of LSD1 and RCOR2 in MKL-1 cells (Fig. 4a and Extended Data Fig. 4). We observed that LSD1 and RCOR2 peaks were enriched in the genes whose expression was perturbed by LSD1 inhibition, such as SMAD9, HEY1 and ID1 (Fig. 4b). RNA-Seq was correlated with LSD1 ChIP-Seq to identify LSD1 targets (1,567 genes) in MCC cell lines (Supplementary Table 5). Remarkably, target genes directly repressed by LSD1 in MCC were enriched in similar pathways to those in which genes were upregulated by EP400 depletion (Extended Data Figs. 1d and 3e,f and Supplementary Tables 6, 7 and 9). These pathways included neuron development, as well as BMP and transcription growth factor-β signalling.

To gain insight into the gene expression program regulated by LSD1 in MCC, we performed motif identification analysis of
**Fig. 1 | MCV small T antigen transactivates components of the LSD1 complex.** a-c, Two biological replicates each of MAX (MAX_1 and MAX_2), EP400 (EP400_1 and EP400_2) and MCV small T antigen (ST_1 and ST_2) ChIP-Seq revealed that MCV small T antigen in a complex with MAX and EP400 binds to the promoters of LSD1 (KDM1A) (a), RCOR2 (b) and INSM1 (c). The University of California Santa Cruz Genome Browser was used to visualize the peaks**. d, RT-qPCR assessment of EP400, RCOR2 and LSD1 levels after the expression of inducible EP400 or control shRNA in MKL-1 cells. The data are presented as the means of biologically independent samples ± s.d. (two-sided t-test). e, ChIP-qPCR indicating that MAX, EP400 and MCV small T antigen (ST) bind specifically to ROR2, LSD1 and ATOH1 promoters in MKL-1 cells. The KRT9 promoter and intergenic region served as negative controls. Immunoprecipitation and mass spectrometry analysis (MudPIT) showing that LSD1 and ROR2 form a multi-protein complex in MCC cell lines. The Venn diagram shows 123 overlapping binding partners of LSD1 and ROR2 from four independent mass spectrometry analyses. g, PHL cells with MCV T antigens (MCV T+) have increased levels of ROR2 and LSD1 mRNA. The INSM1 levels were below detection limits in PHL cells. The signals were normalized to RPLP0. The data are presented as the means of biologically independent samples ± s.d. (two-sided t-test). h, PHL cells with MCV T antigens had increased protein levels of ROR2 and LSD1. Experiments were performed at least three times. i, ROR2 levels increase, and LSD1 binds to ROR2, in PHL cells transformed with MCV T antigens. Immunoprecipitation (IP) of LSD1, followed by western blotting, was performed three times. The hashtag represents a non-specific band. j, ChIP-qPCR of MAX, EP400 and MCV small T antigen in PHL cells expressing T antigens indicated that T antigens enhance MAX and EP400 binding to the ROR2 promoter. The data are presented as the means of biologically independent samples ± s.d. (two-sided t-test). *P < 0.05; **P < 0.005. IgG, immunoglobulin G; LT, MCV large T antigen; VINC, vinculin. Unprocessed western blots are available as source data for h and i.
LSD1 and RCOR2 ChIP-Seq in the MKL-1 cell line. The SeqPos motif tool revealed that the binding motifs of the basic helix–loop–helix transcription factors ATOH1, OLIG2 and ASCL2 were most significantly enriched (Fig. 4c and Extended Data Fig. 4d). ATOH1 is a master transcription factor required for normal Merkel cell lineage. Moreover, ATOH1 was significantly more abundant in...
Fig. 3 | RNA-Seq revealed critical gene expression changes during LSD1 inhibition in MCC. a, MKL-1 cells were treated with GSK-LSD1 (0.1 μM) for 3 d and processed for RNA-Seq (NS: not significant; log2[FC]: fold-change cut-off of 1.5; P: P value cut-off of 10 × 10^-5). The Wald test was performed using the DEseq2 R package with the P values adjusted using the Benjamini–Hochberg method (n = 3 biologically independent samples). b, RNA-Seq of six virus-positive MCC cell lines and one virus-negative UISO cell line treated with LSD1 inhibitors (GSK-LSD1 for 3 d or CPI-670242 for 1 d) (n = 3 biologically independent samples). c, PCA displayed global gene expression changes caused by LSD1 inhibition. d, MKL-1, WaGa and UISO cell lines were treated with GSK-LSD1 (0.05 μM) for 3 d. The signals were normalized to untreated samples and RPLP0 in each sample. The data are presented as the means of 3 biologically independent samples ± s.d. (two-sided t-test; *P < 0.05; **P < 0.005; ***P < 0.0005; ****P < 0.00005). e, LSD1 inhibition increases global levels of H3K4me1 and LSD1 target genes. MKL-1 and WaGa cells were treated with GSK-LSD1 (0.05 μM) for 6 d and the whole-cell lysates and histone extracts were prepared. Experiments were performed at least three times. f, Cells were treated with the LSD inhibitor GSK-LSD1 (0.05 μM) for 3 or 6 d. LSD1 inhibition activates the BMP pathway as assessed by increased levels of phosphorylated SMAD1/5/9 (pSMAD1/5/9). Experiments were performed at least three times. g, Cells were transduced with either control or LSD1-targeting shRNA for 6 d and harvested for western blotting. Experiments were performed at least three times. h, LSD1 inhibition perturbs gene expression in the virus-positive MCC cell lines but not in the virus-negative MCC cell lines. Cells were treated with GSK-LSD1 (0.05 μM) for 3 d. Experiments were performed at least three times. Unprocessed western blots are available as source data for e–h.

virus-positive MCC cells compared with virus-negative cells, while small T antigen, MAX and EP400 bound specifically to the ATOH1 promoter (Fig. 1e and Extended Data Fig. 3i).

ChIP-Seq of ATOH1 in MKL-1 cells revealed that LSD1, RCOR2 and ATOH1 bind to an overlapping set of genes (Fig. 4a,b and Extended Data Fig. 4a–c). ATOH1 binding appeared to be centered on transcription start sites (TSSs) of target genes, while LSD1 and RCOR2 peaks were within 500 base pairs (bp) of the TSSs (Fig. 4d,e). The binding of LSD1, RCOR2 and ATOH1 to proximal promoters (<1 kilobase pair) (15–17%), intragenic regions (~45%)
and distal intergenic regions (~35%) was enriched in similar proportions for each of the three proteins (Fig. 4f and Supplementary Tables 6–8). Pathway analysis of the of LSD1, RCOR2 and ATOH1 peaks revealed that they bind to genes enriched for axon guidance and stem cell pluripotency (Extended Data Fig. 4e).

Next, we sought to determine the effect of LSD1 inhibitors on the DNA occupancy of LSD1, RCOR2 and ATOH1. ChIP-qPCR in untreated MKL-1 cells detected significant enrichment of LSD1, RCOR2 and ATOH1 at the ID1 and SMAD9 promoters. Following LSD1 inhibition, enrichment of LSD1 at these target genes, as well as at ZNF781, HES1 and DLL1, was markedly decreased (Fig. 4g). In contrast, LSD1 inhibition led to increased ATOH1 enrichment at ID1 and SMAD9 (Fig. 4h). This result indicates that LSD1–RCOR2 and ATOH1 compete for binding to a common set of genes.

LSD1 inhibitors have been shown to perturb the neuroendocrine transcription program in small cell lung cancer by disrupting LSD1 interactions with chromatin that are mediated by the SNAG domain protein INSIM1 (ref. 15). In MCC, we determined that LSD1 associates with INSIM1 (Fig. 1f and Supplementary Table 4). We performed immunoprecipitation–mass spectrometry (MudPIT) with an INSIM1 antibody using MKL-1 cells treated with the LSD1 inhibitor (GSK-LSD1) and found that the INSIM1 interaction with LSD1 decreased with inhibitor treatment (Supplementary Table 10). Immunoprecipitation–western blotting experiments confirmed that INSIM1 had reduced binding to LSD1 after the inhibitor treatment (Extended Data Fig. 3g,h).

LSD1 inhibition reduces growth of MCC cells in mice and perturbs neuronal gene expression in human and mouse tissues. To identify changes at the protein level with LSD1 inhibition, we performed multiplexed isobaric tag-based profiling16 of the MKL-1 virus-positive MCC cell line treated with the GSK-LSD1 inhibitor for 8 d. LSD1 inhibition led to major perturbations in protein expression (Fig. 5a). Proteins upregulated by LSD1 inhibition were enriched for pathways in cell adhesion, axonogenesis and neuron differentiation (Extended Data Fig. 5a,b). About 30% of proteins that were significantly increased in response to LSD1 inhibition were also identified as LSD1 targets by the integrated RNA-Seq and ChIP-Seq analysis, including CALR1, PROM1, FAM5B, DLL1 and GFI1 (Extended Data Fig. 3e and Supplementary Table 11).

We tested the efficacy of targeting LSD1 in vivo using MKL-1 and WaGa virus-positive MCC cells grown as xenografts in NOD scid gamma (NSG) mice. When the subcutaneous tumours reached 150 mm³, mice were treated with the CPI-242 LSD1 inhibitor dosed once weekly by oral gavage. LSD1 inhibition significantly decreased the rate of tumour growth in both MCC xenograft models (Fig. 5b and Supplementary Tables 12 and 13).

To explore the tumour response to LSD1 inhibition in vivo, we assessed the proteome of the treated MKL-1 and WaGa xenografts by performing a similar multiplexed isobaric tag-based profiling experiment (Fig. 5c–g, Extended Data Figs. 5c and 6a–d and Supplementary Tables 14–17). We observed that proteins involved in neuron differentiation, including CNTN2 (contactin 2), NEFL (neurofilament light), NEFM (neurofilament medium) and SYT4 (syntaptotagmin 4), were significantly increased in both MKL-1 and WaGa xenografts treated with the LSD1 inhibitor (Fig. 5c). The detection of CNTN2, SYT4, NEFL and NEFM is typically restricted to the brain and some neuronal lineage tissues48. Gene Ontology analysis of the proteins upregulated during LSD1 inhibition was enriched for cytoskeleton organization in neuron projections (Fig. 5d). Interestingly, mouse neuronal proteins, including Pha3a (Rabphilin-3A), Nefl and Dclkl (doublecortin-like kinase 1), also increased during LSD1 inhibition, indicating that LSD1 inhibition affected neuronal differentiation in mouse tissues in the xenograft experiment (Fig. 5f and Extended Data Fig. 5c).

In contrast with the factors upregulated by LSD1 inhibition, proteins with decreased levels were involved in epithelial growth signalling and extracellular organization, including MUC1 (mucin 1), CNPY1 (canopy FGF signalling regulator 1), SPRR1A (small proline-rich protein 1A), CDC6 (cell division cycle 6) and PCOLCE2 (procollagen C-endopeptidase enhancer 2) (Fig. 5e). Mouse proteins that decreased during LSD1 inhibitor treatment of xenografts were enriched for wound healing and inflammatory response pathways, consistent with the observed shrinkage of tumours (Fig. 5g and Extended Data Fig. 5c).

Knockout of ncBAF complex components confers resistance to LSD1 inhibition in MCC. To gain insight into the mechanism of LSD1 inhibition-mediated cell growth inhibition in MCC, we performed a genome-wide CRISPR–Cas9 screen16. MKL-1 cells were transduced with human CRISPR-Cas9 knockout pooled lentivirus libraries followed by treatment with 1.5 nM (the concentration for 30% reduction of cell viability (IC₃₀)) of GSK-LSD1 or vehicle for 20 d. Transduced cells were collected on days 0 and 20 of treatment and sequenced for short guide RNA (sgRNA) (Fig. 6a,b). Reads from day 20 samples (control and treated) were normalized to those from day 0 samples to identify differentially selected genes (Fig. 6c–e and Supplementary Tables 18–20). Knockout of the positively selected genes (group A: 3,624 genes) promoted proliferation during LSD1 inhibition, whereas knockout of the negatively selected genes (group B: 2,207 genes) augmented LSD1 inhibition in blocking cell growth (Fig. 6d). Lysine methyltransferase 2C (KMT2C (MLL3)) was positively selected during LSD1 inhibition (Fig. 6e,f). KMT2C is the primary mammalian H3K4 mono- and dimethyltransferase involved in the activation of cell type-specific gene expression during differentiation51. The positive selection of KMT2C in the resistance screen suggests that it may be a writer of the active histone marks erased by LSD1.

In addition to KMT2C, several components of the ncBAF complex, including BRD9 and GLTSCR1 (BICRA), were among the most significant, positively selected genes in the screen (Fig. 6e,f)16–18. Other significant, positively selected ncBAF components included SMARCD1, SMARCC1 and SMARCA2 (Extended Data Fig. 7a).
To determine whether BRD9 formed the ncBAF complex in MKL-1 cells, we performed large-scale immunoprecipitation of the endogenous BRD9, followed by MudPIT to identify associated proteins (Fig. 6g and Supplementary Table 21). We observed that BRD9 co-precipitated GLTSCR1 and SMARCA4 and several additional components of the ncBAF complex. This finding was confirmed by western blot in MCC cells (Extended Data Fig. 7b). In contrast, BRD9 did not associate with components restricted to the BAF and PBAF complexes, such as ARID1A, ARID2, PBRM1 and BRD7.

LSD1 and BRD9 regulate an overlapping set of genes in MCC.

To assess the role of the ncBAF complex in MCC, we used a bifunctional inhibitor, dBRD9, that selectively degrades BRD9 by bridging the bromodomain and the cereblon (encoded by the CRBN gene) E3 ubiquitin ligase complex (Extended Data Fig. 7d)13. To determine whether depletion of BRD9 confers resistance to LSD1 inhibition, we treated MKL-1 cells with GSK-LSD1 and dBRD9 or a related BRD9 inhibitor (BRD9i; BI-7273) without the linker to CRBN for 6 d, and measured viability by XTT assay (Fig. 7a). The addition of dBRD9 or...
Fig. 5 | Multiplexed isobaric tag-based profiling of the MKL-1 cell line and MCC-derived xenografts revealed that LSD1 inhibition perturbs neuronal gene expression in human and mouse tissues. a. Multiplexed isobaric tag-based profiling of the MKL-1 virus-positive MCC cell line treated with GSK-LSD1 for 8 d displayed global changes in the proteome. Changes in the differentially expressed proteins are shown. Tukey’s honest significance test was performed post hoc on peptide-level linear models for each protein and the P values were adjusted using the Benjamini–Hochberg method. The levels of proteins identified with arrows had a significant fold-change (n = 5 biologically independent samples). Blue represents log2(FC) > 1.5 and FDR < 0.05; pink represents no significant change. b, MKL-1 and WaGa MCC cell lines grown as xenografts in NSG mice had reduced growth rates with LSD1 inhibitors. The hashtag indicates that day 22 data were not plotted as only three out of eight mice were still alive. The data are presented as the means of *n* = 8 independent animals (two-sided t-test; *P* < 0.05; **P* < 0.005; ***P* < 0.0005) (see Supplementary Tables 12 and 13). c–g, Tumours from the MKL-1 or WaGa model (n = 2 independent animals per group per model) were harvested 6 h after the last dose for the TM10plex isobaric tag-labeling quantitative mass spectrometry experiment. The heatmap in c shows the relative abundance of the 30 most upregulated and 30 most downregulated human (tumour) proteins after treatment (see Methods). For d–g, selected GOTERM biological process (BP) and cell compartment (CC) values of –log10[Bonferroni-adjusted P values] for the pathways enriched with the top 100 most differentially expressed upregulated human proteins (d), downregulated human proteins (e), upregulated mouse proteins (f) and downregulated mouse proteins (g) are shown. The clustering test was implemented using kappa statistics**.
**Fig. 6** | CRISPR–Cas9 genome-wide screening for resistance to LSD1 inhibitors creates positive pressure for the loss of ncBAF complex components.  
**a.** Experimental design for CRISPR–Cas9 screening. **b.** MKL-1 cells display sensitivity to GSK-LSD1 in a dose-dependent manner. IC_{50} (1.5 nM) and IC_{10} values (4 nM) are shown. For the CRISPR–Cas9 screen, IC_{30} was used. The data are presented as the means of n = 3 biologically independent samples.  
**c.** MAGeCKFlute normalization results in a normal distribution of scores (selection) for the control and treated (LSD1i) samples (1.5 nM for 20 d).  
**d.** Treatment scores of the control (DMSO; 20 d) and treated samples (LSD1i; 20 d; IC_{30}).  
**e.** Gene ranks based on the scores showed positively and negatively selected genes. The top 20 genes in each group are shown. The dashed lines are the thresholds set to determine the significant differentially selected genes.  
**f.** Top ten most significantly positively selected genes in the LSD1i screen. The P values were estimated using the MAGeCK-MLE model and were adjusted by FDR.  
**g.** Two independent BRD9 MudPIT analyses in MKL-1 cells identified ncBAF complex components, including GLTSCR1 and GLTSCR1L1, that were not included as canonical BAF or PBAF complex-specific components. dNSAF (distributed normalized spectral abundance factor) values are shown (see Methods).
Fig. 7 | LSD1 and BRD9 regulate an overlapping set of genes in MCC. a, BRD9 degradation or inhibition partially rescues the reduced cell viability caused by LSD1 inhibition in MCC. MKL-1 cells were treated with DMSO, LSD1i (0.1 μM), dBRD9 (1 μM), BRD9i (BI-7273; 1 μM) or a combination of LSD1i with dBRD9 or BRD9i for 6 d. The XTT assay was used to measure relative cell viability. The data are presented as the means of n = 3 biologically independent samples ± s.d. (two-sided t-test). b, MKL-1 cells were treated with varying doses of GSK-LSD1 or dBRD9 for 6 d. SynergyFinder was used to calculate negative synergy (rescue) scores. c, RNA-Seq was performed with two replicates of MKL-1 cells treated with DMSO, LSD1i (0.1 μM), dBRD9 (0.1 μM) or both GSK-LSD1 and dBRD9 for 6 d (n = 2 biologically independent samples). The heatmap shows the expression changes in 1,111 genes induced by LSD1i in six virus-positive MCC cell lines (FDR < 0.1; Fig. 3a–c). d, DESeq2 counts of selected LSD1 target genes are shown. Multiple t-tests were performed and the P-values were adjusted by FDR (see Supplementary Table 24). The data are presented as the means of n = 2 biologically independent samples ± s.d. e, Selected GOTERM biological processes, cellular components or Kyoto Encyclopedia of Genes and Genomes (KEGG) −log10(P values) for the pathways enriched with the cluster 1 and 2 genes. The clustering test was implemented using kappa statistics (n = 2 biologically independent samples). f, MKL-1 cells were treated with DMSO, LSD1i (0.05 μM), dBRD9 (1 μM), BRD9i (BI-7273; 1 μM), LSD1i + dBRD9 or LSD1i + BRD9i for 3 d. The signals were normalized to the DMSO-treated sample and RPLPO. Top, cluster 1 pathways; bottom, cluster 2 pathways; gray, KEGG pathway; green, GOTERM cell compartment (CC); orange, GOTERM biological process (BP). The data are presented as the means of n = 3 biologically independent samples ± s.d. P-values were adjusted using the Bonferroni method (two-sided t-test). *P < 0.05; **P < 0.005; ***P < 0.0005; ****P < 0.00005. TGF-β, transforming growth factor-β.
BRD9 partially rescued the decreased levels of cell viability caused by LSD1 inhibition in MKL-1 cells (Fig. 7a,b and Extended Data Fig. 7c).

Since LSD1 inhibition led to the de-repression of specific genes, we suspected that this de-repression required the ncBAF complex. We tested whether simultaneous inhibition of LSD1 and BRD9 could dampen the expression of the LSD1 target genes. We performed RNA-Seq with MKL-1 cells treated with GSK-LSD1 (LSD1i), dBDR9 or both for 6 d (Fig. 7c–e and Supplementary Table 24). PCA of the RNA-Seq data revealed that inhibition of BRD9 or LSD1 caused global changes in gene expression while dual inhibition led to changes distinct from those of either single treatment alone (Extended Data Fig. 7e). The addition of BRD9 degrader significantly dampened the induction of more than half of LSD1 target genes (cluster 1: 622 out of 1,111 genes) (Fig. 7d,e, Extended Data Fig. 8 and Supplementary Tables 22–24).

We validated by RT-qPCR that levels of PROM1, SMARCA1 and CALB2 transcripts increased with LSD1 inhibition, and the addition of dBDR9 or BI-7273 dampened this response (Fig. 7e). Also, the shRNA-mediated knockdown of BRD9 led to decreased levels of PROM1, SMARCA1 and ID1 that were increased by LSD1 depletion (Extended Data Fig. 7f). Genes induced by LSD1 inhibition and repressed by dBDR9 were enriched for pathways involved in neuron differentiation and cell morphology changes (Fig. 7f and Supplementary Tables 25 and 26).

BRD9 is required to de-repress the expression of LSD1 target genes during LSD1 inhibition. We hypothesized that BRD9 is required for increasing levels of LSD1 target genes during LSD1 inhibition. To test this possibility, we performed assay for transposase-accessible chromatin sequencing (ATAC-Seq) to determine the chromatin state of MKL-1 MCC cells treated with LSD1i and BRD9 inhibitors. The PCA analysis of the ATAC-Seq peaks revealed distinct patterns of global changes in chromatin states following LSD1 inhibition, BRD9 degradation or both (Fig. 8a). Remarkably, the combination treatment (LSD1i + dBDR9) had a similar shifting pattern to the control, suggesting that BRD9 degradation restored structural changes in chromatin caused by LSD1 inhibition. We observed that ATAC-Seq peaks reside close to the TSSs in control cells, while LSD1 inhibition increased the open chromatin peaks close to the TSSs, and dBDR9 decreased them (Fig. 8b,c and Extended Data Fig. 9a). By comparing the ATAC-Seq peaks, we determined that the peaks gained by adding dBDR9 to LSD1i reside close to the TSSs whose gene products are involved in pathways such as axon guidance and cell-to-cell communications crucial for differentiation and cancer progression (Fig. 8d,e, Extended Data Fig. 9b–d and Supplementary Table 27). A close examination of the ATAC-Seq peaks in the promoters of LSD1 target genes such as MGP, TNC, ID1, SYT4 and CALB2, the expression of which was dampened by the addition of dBDR9, revealed that LSD1 inhibition increased levels of open chromatin in the promoters, and the addition of dBDR9 dampened this increase (Fig. 8f–i and Extended Data Fig. 9e). These results suggest that BRD9 was required to promote the open chromatin states following LSD1 inhibition.

ChIP-qPCR was performed to assess for LSD1 and BRD9 enrichment on LSD1 target genes in MKL-1 cells (Fig. 8j,k). LSD1 binding to the ID1, ID2, SMAD9 and ZNF781 genes decreased after 3 d of LSD1 inhibition, whereas BRD9 enrichment increased significantly. This observation indicates that as LSD1 left from chromatin, BRD9 (presumably in the ncBAF complex) was recruited to these sites. Together with the ATAC-Seq results, these findings indicate that BRD9 and the ncBAF complex are required for de-repressing the expression of a subset of LSD1 target genes during LSD1 inhibition.

Discussion
Here, we report that MCV small T antigen generates an essential dependence on LSD1 activity through transactivation of LSD1 complex components, leading to repression of multiple LSD1–RCOR2–INSM1 target genes that were in turn activated by the ncBAF complex (Extended Data Fig. 9f). Through a comprehensive targetome analysis integrating RNA-Seq, ChIP-Seq, ATAC-Seq and quantitative mass spectrometry, we established that LSD1 inhibition leads to increased expression of genes involved in neuron differentiation. Our findings support a specific role for LSD1 in repressing BMP signalling, which was previously linked to fate specification of neurons and skin cells48. Our results are consistent with the finding that keratin 14-driven overexpression of BMP in skin dampens Merkel cell development35. LSD1 repression of BMP signalling may also inhibit MCC differentiation that, in turn, regulates neuronal gene expression.

It is important to note that MYCL and the EP400 complex mediate MCV small T antigen oncogenic transformation and LSD1 expression. In this context, the small T antigen–MYCL–EP400 complex represses developmentally related genes by transactivation of the LSD1–RCOR2–INSM1 complex. The MYC and MYCN paralogues of MYCL have been reported to repress specific gene targets by binding directly to a repressor such as MIZ-1 (ZBTB17) or G9a (EHMT2), or by transactivation of a repressor such as EZH2, which in turn represses specific gene targets49,50. Here, we find that MYCL functions to indirectly repress a specific set of developmental genes through the LSD1–RCOR2–INSM1 complex. This activity does not exclude the possibility that MYCL may have additional associated repressor activities mediated by MIZ-1, G9a and EZH2

We observed that DNA bindings of LSD1 and ATOH1 co-localize in proximity to LSD1 target genes. ATOH1 is the master transcription factor for Merkel cell specification26. In this role, ATOH1 may act as a pioneer factor to make active transcription sites accessible for other transcription factors, while LSD1 serves to repress specific genes in these sites based on cellular needs.
We found that all tested virus-positive (but not virus-negative) MCC cell lines responded to LSD1 inhibition. To understand the mechanism of LSD1 inhibition, we determined that the ncBAF chromatin remodelling complex was required to de-repress LSD1 target genes. Inhibition of BRD9—a core component of ncBAF—rescued the loss of cell viability caused by LSD1 inhibition. Loss of ncBAF or its downstream target genes may serve as a mechanism of resistance for targeting LSD1 in neuroendocrine cancers.
The role of ncBAF in cancer is largely unknown. Perturbation of canonical BAF complexes (that is, the SMARC B1 subunit) generates a dependency on BRD9 in synovial sarcoma. In contrast, in cancers with SF3B1 mutations, loss of BRD9 by mis-splicing promotes proliferation, suggesting a tumour suppressor role for BRD9. One promising LSD1 combination therapy may involve immunotherapy. A recent report indicated that LSD1 depletion enhanced programmed death-ligand 1 (CD274) checkpoint blockade in mouse melanoma by increasing type 1 interferon response and anti-tumour T cell infiltration. Historically, therapeutic options for MCC have been limited to surgery, radiation and cytotoxic chemotherapy, although several recent reports showed response rates as high as 62% to checkpoint blockade therapy with programmed cell death protein 1 and programmed death-ligand 1 inhibitors. A strategy combining checkpoint blockade therapy and LSD1 inhibition may prove to be a useful therapeutic strategy for MCC and other cancers.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41556-020-0503-2.

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Methods

Plasmids, cells and cell culture. The expression vectors included pLIX402 inducible empty vector and pLenti CMV Blast empty vector (w3263–1), which were gifts from D. Root (41394; Addgene) and E. Campeau (17486; Addgene), respectively. Lentiviral packaging plasmid pSPAX2 and envelope plasmid pMD2.G were gifts from D. Trono (12260 and 12259; Addgene). The shRNA clone (number 43; CAGGAGAGCTGCTGGTATCA) targeting KDM1A was obtained from Cellecta and was carried in the Cellecta third-generation lentiviral plasmid pKSI-U6wt-sh-CPT-U6-csg-2A-PAgro. Generation of the shRNA clones for EP400 was described by Cheng et al. The shRNA clones targeting R2OR2 (HSsh00209621 and HSsh00209662) and BRD9 (HSsh00209661 and HSsh00206889) were obtained from the Harvard Plasmid Database.

In generating cell lines stably expressing these constructs, IMR90 human lung fibroblast cells and MKL-1 MCC cells were transduced using a three-vector lentivirus transduction system 

MCC cell lines were gifts from M. Shuda (University of Pittsburgh), J. Reeder (Scripps Research Institute), R. Becker (Medical University of Graz) and J. Varadi (University of Wuerzburg). 293T and IMR90 cells were obtained from the American Type Culture Collection. The generation of MKL-1 MCC cell lines with inducible expression of shRNA, and IMR90 transformation using p53DD, MYCL and hTERT constructs were described previously.

The expression vectors included pLIX402 Plasmids, cells and cell culture. The generation of MKL-1 MCC cell lines with inducible expression of shRNA, and IMR90 transformation using p53DD, MYCL and hTERT constructs were described previously.

Antibodies were obtained from Cell Signaling Technology (Ser530), Histone H3 (Lys4) (C64G9) (9725; Cell Signaling Technology; 1:3,000), mono-methyl-histone H3 (Lys4) (61634; Active Motif; 1:2,000) and normal Rabbit IgG (2729; Cell Signaling Technology; 1:1,000). Mouse monoclonal antibodies Ab3 and Ab4 (1:1,000 dilution) were obtained from Sigma. Bead cross-linking with dimethyl pimelimidate. Beads were washed with high-salt wash buffer five times, then eluted with 0.2 M glycine (pH 13) and neutralized with 1 M Tris (pH 8.0). Proteins were precipitated with 1/3 trichloroacetic acid overnight at 4°C, washed with cold acetone twice and analysed by MudPIT as described.

Three additional LSD inhibitors were arrayed in a ten-point dilution series. Maximum concentration before the addition of cells.

Synergy testing was performed using SynergyFinder. Anchorage-independent growth was performed using a ten-step chromatography run. Each full mass spectrometry scan (400–1,400 m/z) was followed by five data-dependent tandem mass spectrometry (MS/MS) scans.

The MS/MS dataset was searched using ProLuCID (version 1.3.3) against a database consisting of 36,628 non-redundant Homo sapiens proteins (downloaded from the National Center for Biotechnology Information RefSeq database on 24 June 2016). 193 usual contaminants and (to estimate false discovery rates (FDRs)) 36,821 randomized amino acid sequences derived from each non-redundant protein entry. To account for alkylation by 2-chloroacetamide (CAM), a carbamidomethylation mass shift (+57 Da) was added stastically to the cysteine residues. To account for the oxidation of methionine to methionine sulfoxide, a 16 Da mass shift was searched as a differential modification. Peptide/ fragment ions were matched to a selected series of precursor and product ion lists, using DTASelect in combination with swallowing (an in-house software package). Original mass spectrometry data can be accessed from the ProteomeXchange Consortium (PXD012156) and MassIVE (MSV00083564) and from the Stowers Original Data Repository (LIFPB-1380).

ChIP-qPCR and RT-qPCR. The ChIP method was modified from protocols described by Schmidt et al. MKL-1 and IMR90 cells were cross-linked using dual cross-linking with disuccinimidyl glutarate and formaldehyde. After cross-linking, the cells were lysed using SimpleChIP buffers A and B (Cell Signaling Technology), and the DNA was processed with micrococcal nuclease (New England Biolabs) for 30 min at 37°C followed by sonication for 20 pulses five times at 4°C. For RT-qPCR, total RNA was purified using an RNeasy Plus Mini Kit (Qiagen), and complementary DNA was synthesized using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems). Quantitative PCR was performed using Brilliant III Ultra-Fast SYBR Green qPCR Master Mix (Agilent Technologies). The primer information can be found in Supplementary Table 28.

Genome-wide CRISPR screening. CRISPR lentiviral libraries H1 and H2 each contain 92,817 pooled sgRNA sequences targeting 18,493 human genes. CRISPR screening was performed by following a previous protocol. Briefly, 2×10^6 MKL-1 cells were transduced with H1 and H2 CRISPR libraries separately at a multiplicity of infection of 0.3 to ensure single sgRNA incorporation per cell. After 6 d of 1 puromycin selection, surviving cells from each sgRNA library transduction were split into three groups, 3×10^4 cells were saved as initial state controls, the remaining two-thirds were divided into two groups and they were treated with either DMSO or an IC_{50} of GSK-LSD1 (1.4 mM; Sigma-Aldrich). These two groups were cultured for 20d, with at least 3×10^4 cells maintained and used as final-state samples. Genomic DNA was extracted and 200 µg from each sample was used to PCR amplify integrated sgRNA sequences and to generate four libraries for next-generation sequencing. Some 50 million reads were obtained for each sequencing library. Copy numbers of every 50 kb segment of the MKL-1 genome were called from the input of ChIP-seq experiments using QDNAseq software. Segmented copy numbers were converted to copy numbers per gene based on gene coordinates. MAGeCK and MAGeCKFlute pipelines were used to assess the significant targets.

Omni-ATAC-Seq. Two independent replicates of 70,000 MKL-1 MCC cells were harvested for ATAC-Seq after treatment with 0.1 µg GSK-LSD1, 0.1 µg dBRD9 and both for 3 d. The cells were washed with PBS twice to achieve >85% viable cells in pellets. The ATAC-Seq protocol was modified from Corces et al. The transposase reaction was performed using TDE1 Trn transposase (15027865; Illumina) and Tuba transducer (15027866; Illumina). The reactions were cleaned up using a MinElute PCR Purification Kit (15229272; Qiagen) and amplified using NEBNext High-Fidelity 2× PCR Master Mix (M0541S; New England Biolabs) for a total of seven cycles. The libraries were cleaned up using the MinElute PCR Purification Kit and additionally with AMPure XP beads (Beckman Coulter) to remove primer dimers and large fragments.
To sequence the libraries, a NextSeq 550 system (Illumina) was used at the Center for Cancer Computational Biology and Molecular Biology Core Facilities following the manufacturer's paired-end sequencing protocol. For analysis, the adapters were removed using Cutadapt27, and the reads were mapped to the hg38 reference using Bowtie 2 (ref. 42) and sorted by SAMtools43. ATAC-Seq peaks were merged, annotated and visualized using BEDtools44 and the ChIPseeker45, ChIPpeakAnno46 and DiffBind47 R packages and Integrative Genomics Viewer (Broad Institute)48.

**Pathway analysis of gene expression.** RNA-Seq of MKL-1 and WaGa MCC cell lines expressing three independent shRNA sequences targeting EP400, an shRNA and a microRNA targeting MYCL, or an shRNA targeting MCV small T antigen, was performed as described previously66. Cells were lysed in 8 M Urea (Thermo Fisher Scientific). The proteins were then reduced and alkylated to Cn, missed cleavages, peptide length, charge state and precursor mass accuracy linear discriminant analysis to an FDR of 1%, as described previously68. XCorr, ΔCn, missed cleavages, peptide length, charge state and precursor mass accuracy were used as parameters for the linear discriminant analysis. The FDR was estimated using a modified method. The target was searched in two databases. The quenched reaction was then combined at a 1:1:1:1:1:1:1:1:1:1 mass ratio, flash dried down in a vacuum centrifuge before resuspension in 10 mM ammonium bicarbonate and 5% acetonitrile for off-line basic pH reversed-phase fractionation. Off-line basic pH reversed-phase high-performance liquid chromatography was performed on an Agilent 1260 pump. A gradient of 13–37% acetonitrile in 10 mM ammonium bicarbonate was used over 50 min. Whole-proteome fractions were collected in a 96-well plate and combined into 24 fractions, of which only non-adherent samples were analysed, as described previously69. The pooled tandem mass tag (TMT)-labelled phosphopeptides were each separated into 96 fractions by the instrument. For each fractionation experiment, fractions were collected in a 96-well plate and combined into 24 fractions as described previously. The 24 fractions were acidified to 1% formic acid and dried down in a vacuum centrifuge. Dried-down fractions were resuspended in 5% acetonitrile and 5% formic acid for liquid chromatography MS/MS (LC-MS/MS) analysis.

**Data for all quantitative TMT LC-MS/MS experiments were collected on an Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific) with liquid chromatography separation performed on an attached Proteome EASY-nLC 1200 liquid chromatography pump (Thermo Fisher Scientific) and CentriCon Centrifugal Filters (Millipore) and method was modified from a previous study. A 100-μm inner-diameter microcapillary column packed with 35 cm of Accucore C18 resin (2.6 μm; 150 Å; Thermo Fisher Scientific) was used to separate the peptides. Approximately 2 μg of peptide was loaded onto the column for analysis. A 150-min gradient of 6–25% acetonitrile in 0.125% formic acid was used at a flow rate of ~450 nl/min49 to separate the peptides from the pooled TMT-labeled samples (MCCs). The fractions were each separated into 96 fractions by the instrument. The 120,000; mass range: 350–1,400 m/z; automatic gain control (AGC) target: 5 × 10^6; maximum injection time: 100 ms). We then used a Top10 method to select precursors for further downstream analysis. MS2 spectra were collected for collision-induced dissociation (AGC target: 5 × 10^6; normalized collision energy: 35%; maximum injection time: 120 ms; isolation window: 0.7 Th). MS2 analysis was performed in the ion trap. We performed an MS3 analysis for each MS2 scan acquired by isolating multiple MS2 fragment ions that were used as precursors for the MS3 analysis with a multi-notch isolation waveform. We detected the MS3 analysis in the Orbitrap (resolution: 50,000) after high-energy collision-induced dissociation (normalized collision energy: 65%; AGC target: 2.5 × 10^6; maximum injection time: 150 ms; isolation window: 1.3 Th).
Statistics and reproducibility. To ensure reproducibility, all of the western blotting experiments were performed at least three times and only the representative data with similar results are reported in the study. Statistical analysis details can be found in the figure captions and Methods.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
ATAC-Seq, ChIP-Seq and RNA-Seq data that support the findings of this study have been deposited in the Gene Expression Omnibus under accession codes GSE124856, GSE124857, GSE124861, GSE124864 and GSE140505. The mass spectrometry data are available at the ProteomeXchange Consortium under accession code PXD012516. All other data supporting the findings and computer codes implemented in this study are available from the corresponding author on reasonable request. Source data for Figs. 1–8 and Extended Data Figs. 1–3 and 7 are presented with the paper.

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Author contributions
D.E.P. and J.A.D. conceived of the study. D.E.P. and J.A.D. developed the methodology. D.E.P., M.Y.L. and L.F. developed the software. D.E.P. and J.C. validated the results. D.E.P., M.Y.L., L.F., C.C., J.P.M. and P.C.G. performed the formal analysis. D.E.P., J.C., J.P.M., M.Y.L., S.K.S. and M.L.T. performed the investigation. J.A.P., P.C.G., M.P.W., P.T. and J.A.D. provided resources. D.E.P. and J.A.D. wrote the original draft of the manuscript. D.E.P., J.C., J.M., M.Y.L., L.F. and J.A.D. reviewed and edited the manuscript. D.E.P. and M.Y.L. visualized the results. P.T., P.C.G., M.P.W. and J.A.D. supervised the study. P.T. and J.A.D. acquired funding.

Competing interests
J.F.M. and P.T. are employees of Constellation Pharmaceuticals. J.A.D. received research funding from Constellation Pharmaceuticals. J.A.D. has served as a consultant to Merck & Co. and EMD Serono.

Additional information
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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Merkel small T antigen transactivates LSD1 complex components. a. Integrated ChIP- and RNA-seq analysis of the MCV ST target genes predicts that MCV ST forms an activator complex. b, RNA-seq results show that EP400 depletion in MKL-1 leads to a reduction in mRNA levels of RCOR2, LSD1, INSM1, and additional components of the LSD1 complex. DESeq2 normalized counts were plotted. Differentially expressed genes were found by comparing each condition with DESeq2 and p-values were adjusted by Benjamini-Hochberg. c, Two independent shRNAs against EP400 decrease levels of EP400 and RCOR2 levels but increase levels of DLL1, HES1, LGR6, KRT17, KRT9, and CDH1 in MKL-1 cells. The RT-qPCR signals were normalized to each uninduced sample and the geometric mean of 36B (RPLP0) and 18s rRNA. d, Depletion of EP400, MYCL, or MCV ST by shRNA leads to increased levels of genes involved in critical cancer and differentiation pathways in MKL-1 and WaGa MCC cell lines (n = 3 independent biological replicates used in each condition). The enrichment test was performed on hypergeometric distribution and the p-values were adjusted by FDR (Supplementary Tables 1–3). e, RNA-seq of MKL-1 cells expressing three independent shRNAs targeting EP400 or ST in MKL-1 and WaGa cells was performed. Mean expression was plotted against log fold change. f, The LSD1 and RCOR2 levels were significantly higher in PHEL (+ MCV LT and wild-type ST) and PH2L (+ MCV LT and the ST mutant E86S-E87S unable to bind the EP400) than in PHL (-MCV T antigens). The fold changes in the western blot signals from four replicate experiments were averaged. Data are shown as mean of n = 4 ± SD; two-sided t-test, *P < 0.05; **P < 0.005. g, A representative blot for Extended Data Fig 1g is shown. The experiment was performed four times. See Unprocessed Gels Extended Data Fig. 1.
Extended Data Fig. 2 | LSD1 inhibition reduces the growth of virus-positive MCC cell lines. a–g. Virus-positive (a–d), but not virus-negative (e–g), MCC cell lines are sensitive to two independent LSD1 inhibitors (GSK2879552 and CPI-670242) in a dose-dependent manner. Relative viability was measured at 12 days of treatment by the CellTiter-Glo assay. Data are shown as mean ± SD and reflect three biological replicates.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Integrative ChIP-seq and RNA-seq LSD1 targetome analysis reveal that LSD1 regulates neuronal differentiation pathways in MCC.  

a, Differential gene expression analysis was performed using DESeq2 and p-values were adjusted by Benjamini–Hochberg. b, c, RT-qPCR (b) and western blot (c) assessment of LSD1 target genes in MKL-1 cells of LSD1 inhibition (GSK-LSD1, 1 μM) in triplicate. For b, the signals were normalized to untreated samples and RPLP0 in each sample. See Unprocessed Gels Extended Data Fig. 3. d, Cells were treated with GSK-LSD1 (0.1 μM) for three days. Data are shown as mean of n = 3 ± SD; two-sided t-test, *P < 0.05; **<0.005. e, Multiplexed Isobaric Tag-Based Profiling of MKL-1 treated with GSK-LSD1 for eight days displays global changes in the proteome. The Venn diagrams show the numbers of genes identified in the RNA- and ChIP-seq-based targetome analysis (Fig. 3; LSD1 MCC targets: upregulated following LSD1i with LSD1 ChIP peaks; RNA down: downregulated following LSD1i) and the proteomics experiment (Supplementary Tables 5 and 11; Proteomics up: upregulated following LSD1i; Proteomics down: downregulated following LSD1i). f, The targetome analysis was performed by integrating ChIP-seq of LSD1 and RNA-seq of the virus-positive MCC cell lines in three independent biological replicates (Supplementary Table 5). Selected GOTERM biological processes (BP) and Cell compartments (CC) –log10 of Bonferroni adjusted p-values are shown. g, The LSD1 IP, followed by western blotting, indicates that LSD1 has reduced binding to INSM1 following LSD1 inhibition. The experiment was performed three times. # non-specific band. See Unprocessed Gels Extended Data Fig. 3. h, IP-western blotting shows INSM1 reduced interaction with LSD1. MKL-1 cells were treated with GSK-LSD1 (0.1 μM) for three days and processed for IP. The experiment was performed at least three times. See Unprocessed Gels Extended Data Fig. 3. i, RT-qPCR of MCC lines and primary human cells (HFF) were performed in triplicate. Data are shown as mean of n = 3 ± SD; two-sided t-test, ***P < 0.0005. # - below detection level.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | ChIP-seq of LSD1, RCOR2, and ATOH1 indicates shared DNA occupancy. 

**a.** Integrated ChIP- and RNA-seq analysis reveals that genes perturbed by LSD1 inhibition are direct LSD1 targets. **b.** Tag heat maps of two replicates of LSD1 (LSD1_1 and LSD1_2), RCOR2 (RCOR2_1 and RCOR2_2), and ATOH1 (ATOH1_1 and ATOH1_2) ChIP-seq show that the factors bind to common regions close to the transcription start sites (TSSs ± 4,000 bp). **c.** The heatmap displays correlations among LSD1, RCOR2, and ATOH1 ChIP-seq peaks. **d.** The SeqPos motif tool was used to determine the LSD1 binding motifs in MKL-1. The binding motifs of ATOH1, as well as OLIG2 and ASCL2, were enriched in the RCOR2 (RCOR2_1) ChIP-seq. **e.** GOTERM biological process pathway analysis of the two independent replicates of LSD1, RCOR2, and ATOH1 ChIP-seq revealed that the factors regulate genes involved in neuronal functions and developmental signaling. The enrichment test was performed on hypergeometric distribution and the p-values were adjusted by FDR.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Multiplexed Isobaric Tag-Based Profiling of MCC-derived xenografts reveals that LSD1 inhibition perturbs neuronal gene expression in human and mouse tissues. a-b, The multiplexed Isobaric Tag-Based Profiling of the MKL-1 virus-positive MCC cell line treated with GSK-LSD1 for eight days identified genes that are differentially expressed during LSD1 inhibition. n = 5 independent biological replicates were used for analysis. Selected GOTERM biological process and cellular compartment terms are shown for the upregulated (a) and downregulated (b) proteins. c, Eight mice were injected with MKL-1 or WaGa MCC cells, and when the tumor size reached 150 mm³, two of each four mice for each MCC model were treated with CPI-670242 (40 mg/kg) orally once a week for 22 days. The tumors were harvested for the TMT-10 plex isobaric tag-labeling quantitative mass spectrometry experiment. c, The heatmap shows the relative abundance of 30 most upregulated and 30 most downregulated mouse proteins after the treatment.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Multiplexed Isobaric Tag-Based Profiling of MCC-derived xenografts profiles global changes in tumor proteomes. The volcano plots display global protein expression changes of MKL-1 human proteins (a), WaGa human proteins (b), MKL-1 mouse proteins (c), and WaGa mouse proteins (d) with or without the LSD1 inhibitor. Tukey’s Honest Significance Test was performed post-hoc on peptide level linear models for each protein and the p-values were adjusted by Benjamini-Hochberg. Each protein is plotted based on its log2 fold change against -log10 of adjusted p-values. The five most upregulated and five most downregulated proteins in each plot are labeled.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | CRISPR screen shows that LSD1 inhibition creates positive pressure for deleting ncBAF complex components. a, Gene ranks based on the differences of the beta (selection) scores between the treatment (GSK-LSD1, 20 days, IC30=1.5 nM) and control (DMSO 20 days) screens show the positively and negatively selected mSWI/SNF components genes including the ncBAF complex components BRD9, GLTSCR1, SMARCA2, SMARCD1, and SMARCC1 (Supplementary Tables 18–20). The rank list contains all the previously reported mSWI/SNF components. b, BRD9 binds to SMARCA4 (BRG1) and GLTSCR1 (BICRA) in MKL-1. IP using a BRD9 antibody followed by western blotting was performed to determine interactions among BRD9, GLTSCR1, and SMARCA4. The experiment was performed at least three times. See Unprocessed Gels Extended Data Fig. 7. c, BRD9 degradation restores the loss of cell viability caused by LSD1 inhibition in MCC. MKL-1 cells were treated with varying doses of the dBRD9 and LSD1 inhibitor (GSK-LSD1) for six days. The XTT assay measured relative cell viability. d, dBRD9 degrades BRD9 efficiently. MKL-1 cells were treated with GSK-LSD1 (0.1 μM), dBRD9 (0.1 μM), or both for three days and harvested for western blotting. The experiment was performed at least three times. See Unprocessed Gels Extended Data Fig. 7. e, The PCA plot shows that the degradation of BRD9 by dBRD9 partially rescues the global gene expression changes caused by LSD1 inhibition. n = 2 independent biological replicates were used for analysis. f, BRD9 depletion by shRNAs rescues gene expression changes caused by LSD1 depletion. MKL-1 cells were transduced with an LSD1-targeting shRNA either with a control shRNA or two distinct BRD9-targeting shRNAs for six days and harvested for western blotting. The experiment was performed at least three times. See Unprocessed Gels Extended Data Fig. 7.
Extended Data Fig. 8 | Heatmaps of gene expression changes following LSD1 and BRD9 inhibition indicate that LSD1 and BRD9 regulate an overlapping set of genes in an antagonistic manner. a, b, RNA-seq was performed with n = 2 biologically independent replicates of MKL-1 cells treated with DMSO, GSK-LSD1 (LSD1i, 0.1 μM), dBRD9 (0.1 μM), or both GSK-LSD1 and dBRD9 for six days. a, Top 50 most differentially expressed genes between LSD1i and LSD1i + dBRD9 are shown. Differential gene expression analysis was performed using DESeq2 and p-values were adjusted by Benjamini-Hochberg. b, All differentially expressed genes (3392 genes; FDR < 0.05) across the conditions are shown.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | ATAC-seq of MKL-1 cells treated with LSD1 and BRD9 inhibitors suggests that BRD9 is required to de-repress a subset of LSD1 target genes. a, Tag heat maps of ATAC-seq peaks show open chromatin regions localize close to the transcription start sites (TSSs, +/- 2000 bp). Combined peaks from two replicates of ATAC-seq of MKL-1 cells treated with DMSO (Control), dBRD9 (0.1 µM), GSK-LSD1 (LSD1i, 0.1 µM), or both dBRD9 and LSD1i (LSD1i_dBRD9) are shown. b–d, The differentially enriched ATAC-seq peaks between Control vs. LSD1i (b), Control vs. dBRD9 (c), and LSD1i vs. LSD1i+dBRD9 (d) are shown with the peak abundance (log concentration) and log fold change in peak scores. The differentially enriched peaks were called using the Diffbind R package with the Wald test and FDR p-value correction. e, ATAC-seq peaks in the promoters of LSD1 target genes ID1, SYT4, and CALB2 with the mentioned conditions are shown. f, Model: Merkel cell polyomavirus activates LSD1-mediated blockade of non-canonical BAF to regulate transformation and tumorigenesis.
## Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see [Authors & Referees](#) and the [Editorial Policy Checklist](#).

### Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

| n/a | Confirmed |
|-----|-----------|
| ☑   | ☑         |

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
  - Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  - Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

*Our web collection on [statistics for biologists](#) contains articles on many of the points above.*

### Software and code

Policy information about [availability of computer code](#)

| Data collection | No software was used to collect data. |
|-----------------|--------------------------------------|
| Data analysis   | STAR 2.5.4a                          |
|                 | HTSeq 0.9.1                           |
|                 | DESeq2                                |
|                 | DAVID Bioinformatics Resources        |
|                 | REVIGO                                |
|                 | Binding and Expression Target Analysis (BETA) |
|                 | BWA 0.7.13                            |
|                 | MACS2 2.1.0                           |
|                 | ChiPseeker 1.22.1                     |
|                 | ChipPeakAnno 3.20.0                   |
|                 | DiffBind 2.14.0                       |
|                 | Integrative genome viewer (IGV; Broad Institute) 2.8.x |
|                 | MAGeCK 0.5.9                          |
|                 | MAGeCKFlute 1.6.3                     |
|                 | GraphPad Prism                        |
|                 | DTASelect                             |
|                 | swalow                               |
|                 | QDNAseq                               |
|                 | Sequest                               |
|                 | ProluCID                              |
|                 | Bowtie2 2.3.4                         |
|                 | ReAdW.exe: Pedrioli, P.G., Eng, J.K., Hubley, R., Vogelzang, M., Deutsch, E.W., Raught, B., Pratt, B., Nilsson, E., Angeletti, R.H., Apweiler, R., et al. (2004). A common open representation of mass spectrometry data and its application to proteomics research. Nature |
For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

**Data**

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE124864
http://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=PXD012516

**Field-specific reporting**

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

**Life sciences study design**

All studies must disclose on these points even when the disclosure is negative.

| Sample size | Sample size of 2-5 for molecular cell biology experiments (RT- and ChIP-qPCR, viability and soft agar colony assays) was determined by power calculation assuming an average effect fold change of 2.5 and standard deviation of 1.0. |
| Data exclusions | No data excluded |
| Replication | Biological experiments were performed independently to ensure reproducibility of the experimental outcomes. The manuscript only contains results that can be reproduced independently. |
| Randomization | Samples were randomly assigned into experimental groups. |
| Blinding | For the soft agar assay, the images were re-named by a colleague in the laboratory and the foci were counted in a blind manner. |

**Reporting for specific materials, systems and methods**

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|---|---|
| n/a | n/a |
| Involved in the study | Involved in the study |
| Antibodies | ChIP-seq |
| Eukaryotic cell lines | Flow cytometry |
| Palaeontology | MRI-based neuroimaging |
| Animals and other organisms | |
| Human research participants | |
| Clinical data | |

**Antibodies**

Antibodies used

- Rabbit polyclonal anti-LSD1 Cell Signaling Technology Cat# 2139; 1:3000
- Mouse monoclonal anti-INSM1 (clone A-8) Santa Cruz Biotechnology Cat# sc-271408; 1:250
- Rabbit polyclonal anti-RCOR2 Proteintech Group Cat# 23969-1-AP; 1:2000
- Rabbit polyclonal anti-ID1 (C-20) Santa Cruz Biotechnology Cat# sc-488; 1:250
- Rabbit polyclonal anti-MYCL1 Proteintech Group Cat# 14584-1-AP; 1:1000
- Rabbit polyclonal anti-PROM1 (CD133) Proteintech Group Cat# 18470-1-AP; 1:3000
- Rabbit polyclonal anti-SMAD9 (aa-200-228) Lifespan Biosciences Cat# LS-C161303-400; 1:1000
- Rabbit monoclonal anti-phospho-smad1(ser463/465)/smad5(ser463/465)/smad9(ser463/467) (clone D5B10) Cell signaling
Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Human lung fibroblast: IMR90 ATCC Cat# CCL-186
Human MCC: MKL-1 (Shuda et al., 2008) Patrick S. Moore’s laboratory at University of Pittsburgh
Human MCC: MKL-2 (Van Gele et al., 2002) Patrick S. Moore’s laboratory at University of Pittsburgh
Human MCC: MS-1 (Shuda et al., 2008) Patrick S. Moore’s laboratory at University of Pittsburgh
Human MCC: UISO (Ronan et al., 1993) Patrick S. Moore’s laboratory at University of Pittsburgh
Human MCC: MCC13 (Leonard et al., 1995) Patrick S. Moore’s laboratory at University of Pittsburgh
Human MCC: MCC26 (Leonard et al., 1995) Patrick S. Moore’s laboratory at University of Pittsburgh
Human MCC: WaGa (Shuda et al., 2008) Jürgen Becker’s laboratory at Medical University Graz, Austria
Human MCC: PeTa (Houben et al., 2013) Jürgen Becker’s laboratory at Medical University Graz, Austria
Human MCC: BroLi (Shuda et al., 2008) Jürgen Becker’s laboratory at Medical University Graz, Austria

Authentication

IMR90 were obtained directly from ATCC.
VP-MCC and VN-MCC lines were confirmed by sequencing (Oncopanel and various RNA-seq experiments). Oncopanel is targeted hybrid capture of 400 genes.

Mycoplasma contamination

All cell lines were negative for mycoplasma contamination.

Commonly misidentified lines

(See ICLAC register)

no commonly misidentified cell lines were used.
Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

| Laboratory animals | Mouse: NSG: NOD.Cg-Prkdcscid Ili2rgtm1Wjl/SzJ The Jackson Laboratory Cat# 005557; Female; 4 to 6 weeks old. |
|--------------------|------------------------------------------------------------------------------------------------------------------|
| Wild animals       | the study did not involve wild animals.                                                                          |
| Field-collected samples | the study did not involve samples collected from the field.    |

Ethics oversight

The animal studies were approved by the DFCI Institutional Animal Care and Use Committee (IACUC).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

May remain private before publication.

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE124864

Enter token ilqtmcmvqjvj into the box

Files in database submission

- LSD1_1_sort_peaks.narrowPeak.bed
- LSD1_2_sort_peaks.narrowPeak.bed
- RCOR2_1_sort_peaks.narrowPeak.bed
- RCOR2_2_sort_peaks.narrowPeak.bed
- ATOH1_1_sort_peaks.narrowPeak.bed
- ATOH1_2_sort_peaks.narrowPeak.bed
- 20160525-LSD1-1-EP3066_S3_R1_001.fastq
- 20160525-LSD1-1-EP3066_S8_R1_001.fastq
- 20160525-LSD1-2-EP3066_S8_R1_001.fastq
- 20160525-LSD1-2-EP3066_S9_R1_001.fastq
- 20160525-RCOR2-1-EP3066_S4_R1_001.fastq
- 20160525-RCOR2-2-EP3066_S9_R1_001.fastq
- 20160525-RCOR2-2-EP3066_S10_R1_001.fastq
- 20160525-Input-1-EP3066_S1_R1_001.fastq
- 20160525-Input-2-EP3066_S6_R1_001.fastq

Genome browser session

(e.g. UCSC)

https://genome.ucsc.edu/s/donglim618/Park_LSD1_RCOR2_ATOH1_ChIPseq

Methodology

Replicates

There are two biologically and technically independent ChIP replicates. Data for each replicate (not combined) are shown in figures.

Sequencing depth

- LSD1_1:
  - tag size is determined as 75 bps
  - total tags in treatment: 39138041
  - tags after filtering in treatment: 36570635
  - maximum duplicate tags at the same position in treatment = 1
  - Redundant rate in treatment: 0.07
  - total tags in control: 41807511
  - tags after filtering in control: 38806784
  - maximum duplicate tags at the same position in control = 1
  - Redundant rate in control: 0.07
  - d = 146

- LSD1_2:
  - tag size is determined as 75 bps
  - total tags in treatment: 49000665
  - tags after filtering in treatment: 45049086
  - maximum duplicate tags at the same position in treatment = 1
  - Redundant rate in treatment: 0.08
  - total tags in control: 42349718
  - tags after filtering in control: 39249637
  - maximum duplicate tags at the same position in control = 1
  - Redundant rate in control: 0.07
  - d = 146

- RCOR2_1:
Antibodies
Rabbit polyclonal anti-LSD1  Cell Signaling Technology Cat# 2139
Rabbit polyclonal anti-RCOR2  Proteintech Group Cat# 23969-1-AP
Rabbit polyclonal anti-ATOH1 Proteintech group Cat# 21215-a-AP

Peak calling parameters
Peaks were called using using the default parameters (band width = 300, model fold = [5, 50], qvalue cutoff = 1.00e-02 and tag size is determined as 75 bps)of MACS2 v2.1.0 relative to the input samples. .narrowPeak.bed files were generated using MACS2 v2.1.0.

Data quality
With the qvalue cutoff = 1.00e-02,
LSD1_1: 68197 peaks
LSD1_2: 30688 peaks
RCOR2_1: 62798 peaks
RCOR2_2: 33899 peaks
ATOH1_1: 106317 peaks
ATOH1_2: 86123 peaks

Software
ChIPseeker
ChIPpeakAnno
DiffBind
Integrative genome viewer (IGV; Broad Institute)