

An EZH2–SREBP2 axis promotes cholesterol biosynthesis and represents a noncanonical vulnerability in tumorigenesis

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Enhancer of zeste homolog 2 (EZH2) is frequently overexpressed in cancer, correlating with adverse clinical outcomes. However, how EZH2 overexpression supports tumorigenicity is not fully elucidated. Here we identify a previously unexplored tumour-promoting axis involving EZH2–SREBP2 association. EZH2 and SREBP2, a master regulator of lipid metabolism, cooperate to drive high expression of mevalonate pathway genes, enhancing cholesterol biosynthesis and sustaining tumour growth. Transcriptional activation domains of EZH2 and SREBP2 bind p300 directly, mediating proto-oncogene activation. Furthermore, we employed proteolysis targeting chimeras (PROTACs) to target this non-canonical EZH2 function. Independent EZH2-targeting PROTACs degrade both EZH2 and SREBP2, downregulating SREBP2-associated gene-expression programmes and inhibiting tumour growth. Collectively, this study unveils an EZH2–SREBP2 regulatory axis that promotes cholesterol biosynthesis and fuels tumorigenesis, shifting the current paradigm of EZH2's oncogenic functions.

Cancer hallmarks, including epigenomic, transcriptomic and metabolic dysregulations, often intersect to drive oncogenesis^{1–3}. Enhancer of zeste homolog 2 (EZH2; also known as lysine methyltransferase 6A or KMT6A) is an epigenetic regulator closely linked to tumorigenesis. Traditionally viewed as the enzymatic subunit of Polycomb repressive complex 2 (PRC2), EZH2 silences tumour suppressor genes (TSGs) by catalysing histone H3 lysine 27 trimethylation (H3K27me3)^{4,5}. Driven partly by the genomic loss of EZH2-targeting microRNAs or *EZH2* gene amplification, EZH2 is frequently overexpressed in malignancies^{6,7}, such as prostate cancer⁶, liver tumour⁸ and B cell lymphoma⁹.

This overexpression correlates with disease progression and poor prognosis^{8–10}. Beyond its canonical role, increasing evidence indicates that EZH2 possesses non-conventional gene-activation functions in cancer^{11–18}. Despite these advances, how EZH2 drives tumorigenesis remains elusive in many unexplored contexts.

Using unbiased genomic profiling, we demonstrate that EZH2 non-canonically interacts with sterol regulatory element (SRE)-binding protein 2 (SREBP2; also known as SREBF2), a master transcription factor (TF) of lipid metabolism¹⁹, to maintain cholesterol biosynthesis and homeostasis in cancer. Mechanistically, multivalent interactions

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between the partially disordered transcriptional activation domains (tADs) of EZH2 and SREBP2 and the co-activator p300 coordinate to activate mevalonate (MVA) pathway genes²⁰, fuelling tumour cell survival and proliferation. This previously unstudied EZH2–SREBP2–MVA axis is conserved across multiple malignancies, including liver cancer, B cell lymphomas (typically considered to be driven by canonical EZH2:PRC2 hyperactivation^{9,21}), androgen receptor (AR)-positive prostate cancer and MLL-rearranged (MLL-r) acute leukaemia. To block this non-canonical oncogenic activity of EZH2, we employed proteolysis targeting chimeras (PROTACs)²². Notably, independent EZH2 PROTACs degrade both EZH2 and its functional partner SREBP2 through a bystander effect, exerting potent growth inhibition in EZH2- or SREBP2-dependent cancers. These effects were not observed with conventional EZH2 enzymatic inhibitors, highlighting a unique therapeutic advantage of EZH2 PROTACs.

Collectively, this study uncovers a critical role for the EZH2–SREBP2 axis in coordinating epigenomic, transcriptomic and metabolic states to drive oncogenesis. Moreover, our findings establish a promising therapeutic strategy to target this axis.

Results

EZH2 non-canonically binds genomic sites associated with gene-activation markers in liver cancer

Liver cancer is the third leading cause of cancer death worldwide²³. Using publicly available TCGA datasets, we initially found that *EZH2* expression is significantly increased in hepatocellular carcinoma (HCC) samples compared with matched healthy tissues (Extended Data Fig. 1a). Using western blot (WB), we also verified elevated EZH2 protein levels relative to adjacent healthy tissues (Extended Data Fig. 1b). Furthermore, higher *EZH2* expression correlates with worse patient survival, suggesting an oncogenic role (Extended Data Fig. 1c). Indeed, knockdown (KD) of *EZH2* in the Huh7 HCC cells significantly inhibited proliferation detected via colony-formation and viability assays (Extended Data Fig. 1d–f). HepG2 cells, a hepatoblastoma (HB)-derived line that resembles HCC²⁴, also exhibited EZH2-dependent growth (Extended Data Fig. 1d,g,h).

To dissect the gene-regulatory functions of overexpressed EZH2 in liver cancer, we performed cleavage under targets and release using nuclease (CUT&RUN) for EZH2 and H3K27me3, the histone mark characteristic of PRC2:EZH2 activity. To ensure rigour, we used independent cell models (Huh7 and HepG2) and, additionally, generated cells stably expressing HA-tagged exogenous EZH2. The CUT&RUN profiles of endogenous EZH2 and exogenous HA-tagged EZH2 highly correlated with each other (Extended Data Fig. 1i). Independent H3K27me3 antibodies also yielded highly consistent profiles (Extended Data Fig. 1j). Unsupervised clustering revealed that EZH2 not only overlaps H3K27me3 at expected EZH2:PRC2-targeted sites (EZH2_ensemble sites) but also exhibits ‘solo’ binding at sites lacking H3K27me3 (EZH2_solo sites; Fig. 1a–d). EZH2-solo sites account for approximately 80% of sites in both models (Fig. 1a,c; top) and yet they have not been explored in liver cancer. Notably, EZH2-solo peaks co-localize with prominent gene-activation-related markers, including

histone acetylation, H3K4me3, p300 and RNA polymerase II (Pol II), which are generally absent from PRC2-targeted EZH2-ensemble sites (Fig. 1a–f). Furthermore, CUT&RUN using a primary HCC sample exhibiting EZH2 overexpression (Extended Data Fig. 1b) defined similar EZH2-solo sites (Extended Data Fig. 1k–l), demonstrating considerable overlap with the cell line results (Fig. 1g).

Together, the EZH2 regulome in liver cancer extends well beyond its canonical role. EZH2-solo binding in liver cancer is clinically relevant, yet it has not been studied to date.

In liver cancer, EZH2 co-localizes with SREBP2 at EZH2-solo peaks, enriched for mevalonate (MVA) pathway genes

To delineate the genomic features unique to EZH2-solo sites, we conducted unbiased motif search analyses and found that EZH2-solo peaks are enriched for the consensus motif of SREBP2, a master regulator of cholesterol metabolism^{19,20}, in both Huh7 and HepG2 cells (Fig. 1h). Under low-sterol conditions, SREBP2, synthesized as an inactive precursor in the endoplasmic reticulum (ER), is escorted to the Golgi and proteolytically processed, producing a truncated SREBP2 that subsequently enters the nucleus as nuclear SREBP2 (n-SREBP2; Extended Data Fig. 1m)^{19,25}. n-SREBP2 binds SREs of target genes^{19,25}. Motifs of NF-Y and Sp1, the TFs known to cooperate synergistically with n-SREBP2 (ref. 26), were also enriched at EZH2-solo sites (Extended Data Fig. 1n).

To further assess the association between EZH2 and n-SREBP2, we conducted CUT&RUN for n-SREBP2 and detected its enrichment at EZH2-solo sites in both Huh7 (Fig. 1i,j) and HepG2 cells (Fig. 1k–l). In fact, the majority of the SREBP2-bound peaks co-localized with EZH2-solo binding sites (Fig. 1m). n-SREBP2 potentiates cholesterol homeostasis via MVA pathway genes^{20,27}. Of note, all MVA pathway genes (including *HMGCS1*, *HMGCR*, *MVK*, *MVD*, *ID11*, *FDF1*, *MSMO1* and *EBP*) are co-bound by EZH2, n-SREBP2, p300, Pol II and gene-active histone marks (such as H3K27ac) and not the repressive H3K27me3 mark (Fig. 1n and Extended Data Fig. 1o). This pattern sharply differs from classic PRC2:EZH2-targeted genes (such as *IGFBP2*; Extended Data Fig. 1p), which are marked by EZH2 and H3K27me3 but lack gene-active histone marks, p300 and n-SREBP2. Co-immunoprecipitation (IP) detected EZH2 binding to n-SREBP2 using endogenous or exogenous n-SREBP2 (Fig. 1o–p and Extended Data Fig. 2a). Notably, n-SREBP2 associates with EZH2 but not other PRC2 components, EED and SUZ12 (Fig. 1p), consistent with n-SREBP2 enrichment at EZH2-solo sites. Co-IP also confirmed association of both SREBP2 and EZH2 with p300 using endogenous (Fig. 1o) and exogenous protein (Extended Data Fig. 2b–d).

Collectively, EZH2 non-canonically interacts with n-SREBP2 and the two exhibit genome-wide co-localization in liver cancer, notably at MVA pathway genes.

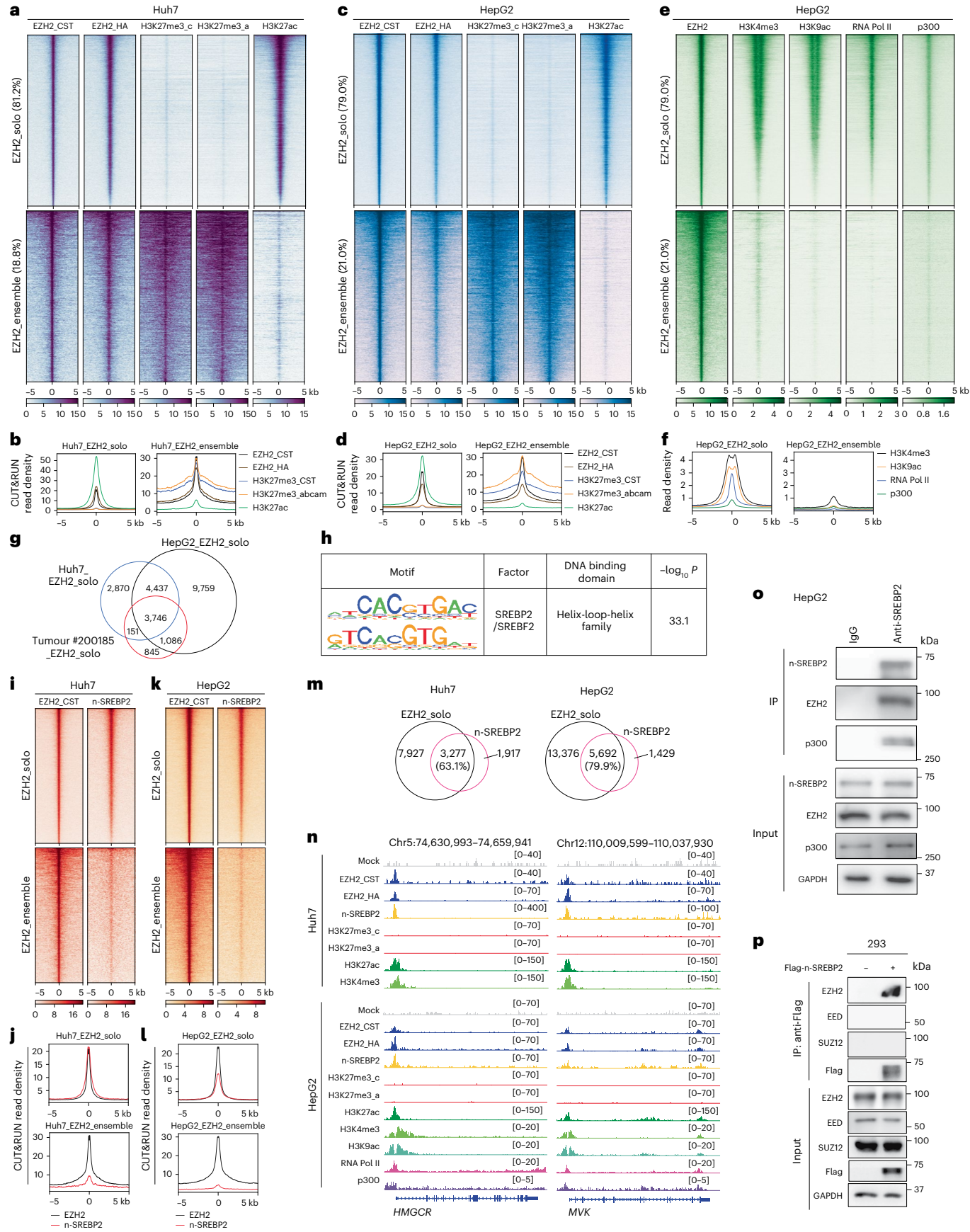
EZH2 and SREBP2 act together to drive transcription of MVA pathway genes, promoting tumour cell growth

To delineate the EZH2-controlled molecular pathways in liver cancer, we conducted RNA sequencing (RNA-seq) in Huh7 and HepG2 cells following *EZH2* KD versus controls (Supplementary Table 1 and

Fig. 1 | EZH2 non-canonically interacts with n-SREBP2, exhibiting genome-wide co-localization at mevalonate (MVA) pathway genes in liver cancer cells.

a–d, Heatmaps (**a,c**) and averaged signal intensities (**b,d**) of CUT&RUN peaks for EZH2 (using endogenous EZH2 antibody and HA antibody for HA-EZH2), H3K27me3 (using Cell Signaling Technologies (H3K27me3_c) and Abcam (H3K27me3_a) antibodies) and H3K27ac, ± 5 kb from EZH2 peak centres in Huh7 (**a,b**) or HepG2 cells (**c,d**). Unsupervised *k*-means clustering was used to define canonical EZH2⁺/H3K27me3⁺ peaks (EZH2-ensemble) and non-canonical EZH2⁺/H3K27me3[−] peaks (EZH2-solo), shown in the bottom and top of **a** and **c**, respectively. **e,f**, Heatmaps (**e**) and averaged signal intensities (**f**) showing binding of the indicated proteins, ± 5 kb from EZH2-solo or EZH2-ensemble peak centres in HepG2 cells. **g**, Venn diagram illustrating the overlap between

EZH2-solo peaks identified in human liver cancer lines (Huh7 and HepG2) and a primary HCC sample. **h**, Enrichment of the SREBP2 consensus motif at EZH2-solo sites. **i–l**, Heatmaps (**i,k**) and averaged signal intensities (**j,l**) showing CUT&RUN peaks of n-SREBP2, ± 5 kb from EZH2-solo or EZH2-ensemble peak centres in Huh7 (**i,j**) and HepG2 cells (**k,l**). **m**, Venn diagram showing overlap between n-SREBP2 and EZH2-solo binding sites in Huh7 and HepG2 cells. **n**, Integrative Genomics Viewer (IGV) tracks showing genomic enrichment of the indicated proteins at *HMGCR* and *MVK* in Huh7 and HepG2 cells. **o**, Co-IP of endogenous EZH2, n-SREBP2 and p300 in HepG2 cells. **p**, Co-IP of endogenous PRC2 and Flag-tagged n-SREBP2 in HEK293 cells using anti-Flag antibodies. Unprocessed blots are available in the Source data file.



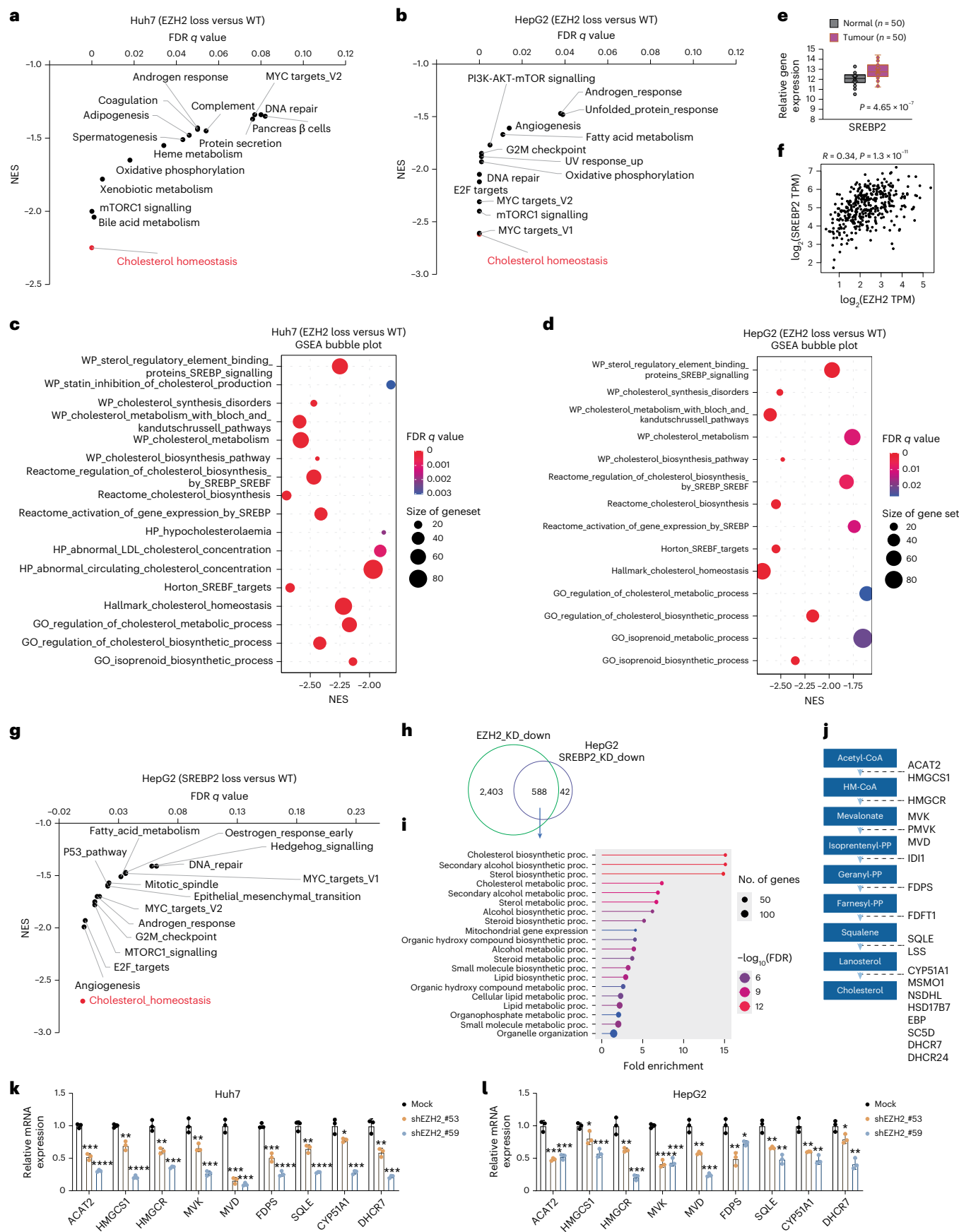


Fig. 2 | EZH2 and SREBP2 both potentiate the expression of MVA pathway genes, promoting tumour cell growth. **a, b**, Summary plots showing negative enrichment of the indicated hallmark pathways in Huh7 (**a**) or HepG2 (**b**) cells following EZH2 KD versus WT controls based on RNA-seq. NES, normalized enrichment score. **c, d**, Bubble plots showing gene set enrichment based on GSEA of Huh7 (**c**) or HepG2 (**d**) cells following EZH2 KD versus WT controls. The x axis indicates NES, while the colour and size of bubbles represent the false discovery rate (FDR) *q* value and gene set size, respectively. **e**, Box plot showing *SREBP2* expression levels in primary tumours versus matched normal tissues of the TCGA HCC patient cohort (*n* = 50). Data represent normalized counts (median $\pm$ s.e.m.; box boundaries indicate 25th–75th percentiles, centre lines indicate median and whiskers indicate 1.5 $\times$ the interquartile range). *P* value calculated by an unpaired, two-tailed Student's *t*-test. **f**, Pearson correlation between *EZH2* and *SREBP2* transcription levels in TCGA HCC tumours. **g**, Summary plot showing

negative enrichment of the indicated hallmark pathways in HepG2 cells following *SREBP2* KD versus WT controls. **h, i**, Venn diagram (**h**) showing overlap of DEGs downregulated following KD of either *EZH2* or *SREBP2* in HepG2 cells and GO biological process analysis (**i**) of 588 genes co-activated by EZH2 and SREBP2. The threshold for DEGs was set at an adjusted *P* (Padj) value < 0.05 and fold change (FC) > 1.50, based on RNA-seq (*n* = 2 independent experiments). **j**, Schematic of cholesterol biosynthesis, with critical MVA pathway genes labelled on the right. **k, l**, RT–qPCR for the indicated MVA pathway genes in Huh7 (**k**) or HepG2 (**l**) cells following EZH2 KD versus mock (*n* = 3 independent experiments). Data represent relative mRNA levels (mean $\pm$ s.d.) normalized to an internal control and to mock-treated samples. *P* values were calculated by an unpaired, two-tailed Student's *t*-test (**P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001). Source data are provided in the Source data file.

Extended Data Fig. 2e, f). As expected, *EZH2* depletion correlated with the reactivation of genes suppressed by PRC2 and H3K27me3 in both models, as assessed by gene set enrichment analysis (GSEA) (Extended Data Fig. 2g, h) and quantitative PCR with reverse transcription (RT–qPCR) (Extended Data Fig. 2i, j). Meanwhile, GSEA of hallmark genes showed that transcripts involved in cholesterol homeostasis were among the most significantly downregulated in *EZH2*-depleted versus wild-type (WT) cells (Fig. 2a, b). Indeed, closer examination of lipid metabolism genes showed that n-SREBP2-mediated cholesterol biosynthesis and the MVA pathway are positively associated with EZH2 expression in both lines (Fig. 2c, d and Extended Data Fig. 2k, l). These observations point to a previously unknown requirement for EZH2 in activating n-SREBP2-targeted cholesterol biosynthesis genes, which agrees with the co-localization of EZH2-solo and n-SREBP2 at genomic sites carrying gene-active markers (Fig. 1i–n). Thus, EZH2 has dual regulatory functions, acting as a co-repressor at canonical EZH2:PRC2 targets, while serving as a co-activator at non-canonical targets co-bound by EZH2-solo and n-SREBP2.

Akin to *EZH2*, *SREBP2* expression is significantly increased in tumours of TCGA patients with HCC compared with healthy tissues (Fig. 2e). Moreover, *SREBP2* and *EZH2* expression levels positively correlate with one another (Fig. 2f), suggesting their co-activation during disease progression. To assess the role of SREBP2 in HCC, we depleted *SREBP2* in HepG2 cells using independent short-hairpin RNAs (shRNAs) (Extended Data Fig. 2m) and found that *SREBP2* depletion resembles *EZH2* loss, leading to significantly decreased proliferation (Extended Data Fig. 2n, o). Subsequent RNA-seq identified 630 transcripts significantly downregulated upon *SREBP2* KD relative to controls (Supplementary Table 2). As expected, these transcripts are enriched for cholesterol biosynthesis and lipid metabolism genes (Fig. 2g and Extended Data Fig. 2p). Of note, a vast majority

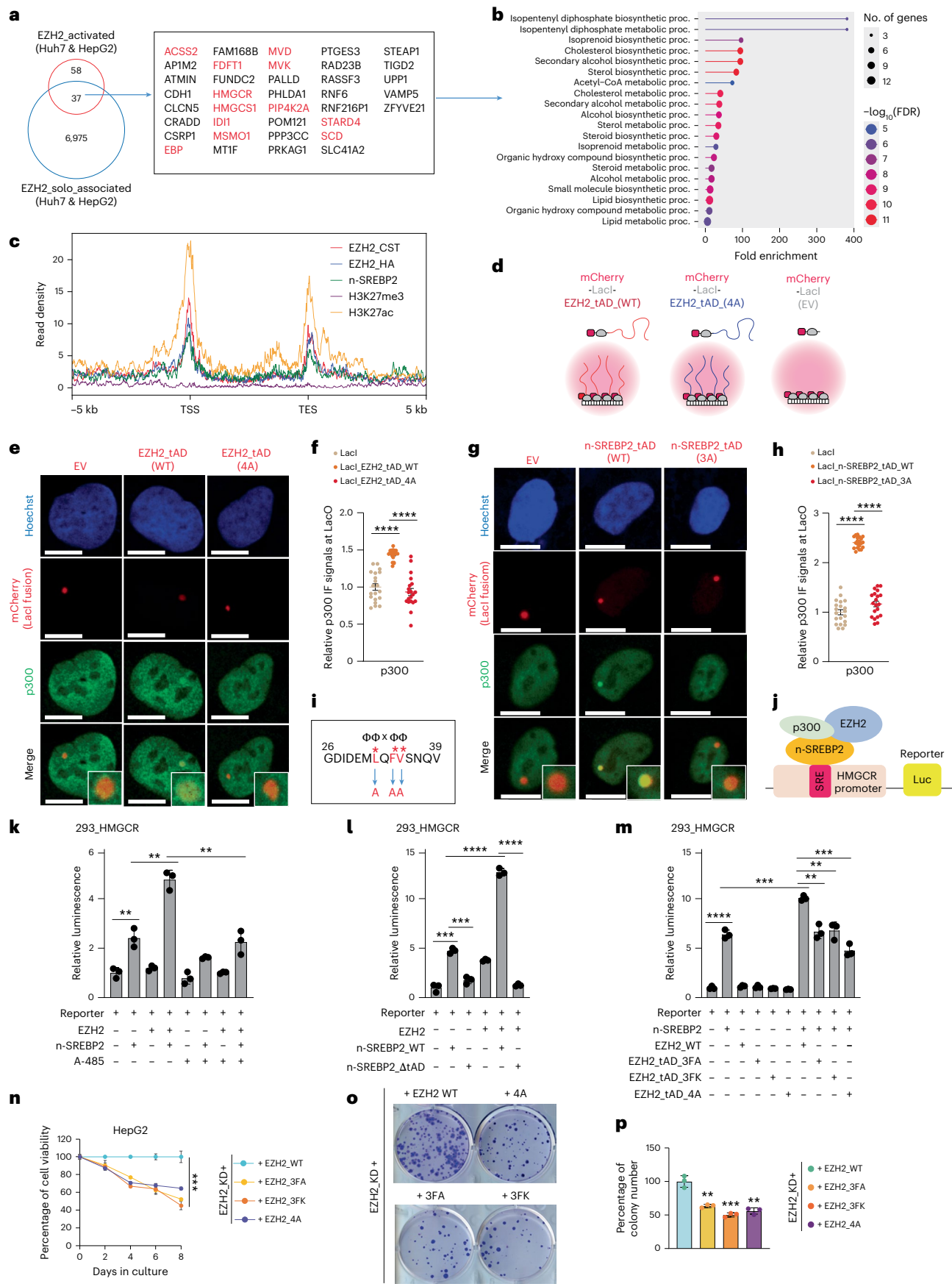
(588 out of 630) of these n-SREBP2-activated genes exhibited EZH2 co-dependency for transcription (Fig. 2h). Gene Ontology (GO) analysis of these EZH2:n-SREBP2-co-activated transcripts again showed the greatest enrichment for cholesterol biosynthesis and metabolism genes (Fig. 2i). Cholesterol metabolism and its derived metabolites (Fig. 2j) crucially support cancer progression^{28–30}. RT–qPCR following *EZH2* KD with independent shRNAs confirmed requirement of EZH2 for activating MVA pathway genes, such as *ACAT2*, *HMGCS1*, *HMGCR*, *MVK* and *MVD*, in both Huh7 and HepG2 cells (Fig. 2k, l). In contrast, depletion of *EED*, a PRC2 component that interacts with EZH2, did not downregulate MVA pathway genes (Extended Data Fig. 2q, r), again supporting the notion that EZH2-mediated activation of MVA pathway genes is PRC2-independent.

The tADs of EZH2 and n-SREBP2 directly bind p300, promoting transcription of cholesterol biosynthesis genes

Having observed a PRC2-independent function of EZH2 in liver cancer, we next aimed to define the non-canonical targets directly activated by EZH2. Integrating genomic datasets from Huh7 and HepG2 cells, we identified 37 genes that exhibit EZH2-solo binding and are activated by EZH2 in both models (Fig. 3a and Supplementary Table 1). This non-canonical EZH2 target signature is highly enriched for transcripts crucial for cholesterol biosynthesis and the MVA pathway, such as *HMGCS1*, *HMGCR*, *MVK* and *MVD*, as well as genes crucial for cholesterol processing, such as *ACSS2* (ref. 31), *STARD4* (ref. 32), *SCD*³³ and *PIP4K2A*³⁴ (Fig. 3a, b). Utilizing binding and expression target analysis (BETA)³⁵, we further observed significant associations of EZH2-solo binding with gene activation in HepG2 and Huh7 cells, albeit with a mild effect at the genomic scale (Extended Data Fig. 3a). We thus focused on top-ranked targets based on BETA rank-based plots and found that genes controlling cholesterol metabolism are directly activated

Fig. 3 | The tADs of EZH2 and n-SREBP2 bind p300, promoting the transcription of cholesterol metabolism genes. **a, b**, Venn diagram (**a**) identifying EZH2-solo-targeted genes downregulated upon EZH2 KD versus mock in both Huh7 and HepG2 cells (with cholesterol metabolism genes highlighted in red) and subsequent GO analysis (**b**). **c**, Averaged intensities of EZH2, n-SREBP2, H3K27me3 and H3K27ac CUT&RUN signals at the 37 targets directly activated by EZH2 in HepG2 cells as identified in **a**. TSS, transcription start site; TES, transcription end site. **d**, Schematic showing the LacO array bound by mCherry–LacI fused to the EZH2-tAD, either WT (left) or 4A mutant (middle), with mCherry–LacI alone (right) used as a control in U2OS 2–6–3 cells. **e–h**, mCherry–LacI fusion (mCherry; red) and p300 immunofluorescence (IF; green) imaging to assay their interaction in situ in U2OS 2–6–3 cells (**e, g**) and quantification of relative IF signals of p300 in mCherry–LacI foci, either alone or fused to the tAD of EZH2 (**f**) or n-SREBP2 (**h**), either WT or tAD-defective. Insets in the bottom of **e** and **g** show the magnified mCherry–LacI fusion bound to the LacO array. Scale bar, 10 μ m. The y axis in **f** and **h** shows relative IF signals (mean $\pm$ s.e.m.) normalized to LacI controls (*n* = 20 cells). *P* values were calculated by an unpaired, two-tailed Student's *t*-test (*****P* < 0.0001). **i**, Aromatic residues in the SREBP2 tAD mutated to alanine (termed 3A). **j**, Schematic of

transactivation assays using a luciferase reporter carrying the proximal *HMGCR* promoter. **k–m**, *HMGCR*-luciferase reporter activity following transfection of n-SREBP2, EZH2 or their combination into 293 cells. Assays were performed either in the absence or presence of the p300/CBP inhibitor A-485 (**k**), using WT versus tAD-deleted n-SREBP2 (**l**), or using WT EZH2 versus the 3FA, 3FK or 4A mutant form of tAD (**m**). Relative luminescence was normalized to Renilla internal controls and mock-transfected cells (*n* = 3 independent experiments; mean $\pm$ s.d.). *P* values were calculated by an unpaired, two-tailed Student's *t*-test (***P* < 0.01; ****P* < 0.001; *****P* < 0.0001). **n**, Proliferation of EZH2 KD HepG2 cells rescued with either WT EZH2 or tAD-defective mutant (*n* = 3 independent experiments). Data represent relative growth (mean $\pm$ s.d.) normalized to WT EZH2-rescued cells. *P* values were calculated by an unpaired, two-tailed Student's *t*-test (****P* < 0.001). **o, p**, Representative images (**o**) and quantification (**p**) of colony formation by HepG2 cells following the rescue of EZH2 KD with either WT EZH2 or its tAD-dead mutant. Data represent colony quantification (mean $\pm$ s.d.; *n* = 3 independent experiments). *P* values were calculated by an unpaired, two-tailed Student's *t*-test (***P* < 0.01; ****P* < 0.001). Unprocessed images, source numerical data and *P* values are available in the Source data file.



by EZH2 (Supplementary Table 3 and Extended Data Fig. 3b–e). Non-canonical targets of EZH2 are characterized by the co-binding of n-SREBP2, gene-active histone marks, RNA Pol II and p300, but lack H3K27me3 (Fig. 3c and Extended Data Fig. 3f).

EZH2's cryptic, partially disordered tADs (Extended Data Fig. 3g) use conserved hydrophobic motifs to directly bind the CH3 domain of p300, a histone acetyltransferase (HAT)^{17,36,37}. Likewise, n-SREBP2's tAD binds p300 (ref. 38). Using recombinantly expressed proteins, we detected direct protein–protein interactions between the p300 CH3 domain and the tADs of EZH2 and n-SREBP2 (Extended Data Fig. 3h,i), pointing to a multivalent association. Furthermore, we reconstituted a high local concentration of EZH2's tAD at a specified genomic locus in cells to ask whether it is sufficient to recruit regulatory partners. We fused EZH2's tAD to mCherry–LacI, followed by its expression in U2OS 2-6-3 cells containing a large integrated LacO array^{39–41} (Fig. 3d, left). This system also allows us to define whether mutated EZH2 tADs recruit a co-activator under the same well-controlled context (Fig. 3d, middle). Into the Lac array-containing cells, we transfected either mCherry–LacI alone or mCherry–LacI fused to WT or a functionally defective mutant of EZH2's tAD (Extended Data Fig. 3g; WT versus a mutant termed 4A, in which four hydrophobic residues are mutated to alanine), followed by co-immunostaining for p300 (Fig. 3e). Subsequent imaging revealed significant enrichment of p300 within the reconstituted foci containing the WT EZH2-tAD, but not LacI alone or the 4A-mutant tAD (Fig. 3e,f). Using this strategy, we readily captured p300 interaction with the WT tAD of n-SREBP2 and not a tAD-defective form⁴² (Fig. 3g–i; WT versus 3A).

Next, we turned to a luciferase reporter carrying the proximal promoter of *HMGR*, a direct target of EZH2-solo and n-SREBP2 (Fig. 3j). n-SREBP2 activated transcription from this reporter, an effect enhanced by co-expressed EZH2 (Fig. 3k and Extended Data Fig. 3j). Treatment with A-485, an enzymatic inhibitor of p300, or siRNA-based p300 depletion significantly blocked n-SREBP2:EZH2-induced reporter activation (Fig. 3k and Extended Data Fig. 3j). In agreement, treatment of Huh7 and HepG2 cells with CBPD-268 (Extended Data Fig. 3l,m), a p300 degrader⁴³, or A-485 (Extended Data Fig. 3n) significantly downregulated MVA pathway genes, supporting a requirement for p300's intrinsic HAT activity in SREBP2:EZH2-dependent activation. Furthermore, we assessed the roles for the tADs of EZH2 and n-SREBP2 during reporter activation. Compared with WT, the tAD-deleted mutant of n-SREBP2 failed to activate the *HMGR* reporter (Fig. 3l); likewise, none of the tested EZH2-tAD mutated forms (Extended Data Fig. 3g) enhanced n-SREBP2-mediated reporter activation (Fig. 3m). We also conducted *EZH2* rescue in *EZH2*-depleted liver cancer cells and found that, compared with WT, *EZH2*-tAD mutants showed impaired proliferation and colony formation (Fig. 3n–p and Extended Data Fig. 3o), resembling depletion of *EZH2* (Extended Data Fig. 1e–h) or *SREBP2* (Extended Data Fig. 2n,o).

Altogether, these results support the notion that partially disordered tADs of EZH2 and n-SREBP2 directly bind p300 to activate a suite of genes controlling cholesterol metabolism and processing, sustaining malignant growth.

EZH2-targeting PROTACs uniquely degrade both EZH2 and SREBP2

EZH2 enzymatic inhibitors, including EPZ-6438/tazemetostat⁴⁴ and C24 (ref. 17), have limitations in cancer treatment, primarily due to

their failure to block EZH2's methyltransferase-independent functions. Previously, we discovered MS177, an EZH2-targeting PROTAC that depletes EZH2 in MLL-rearranged acute myeloid leukaemia (MLL-r AML)¹⁷, which we confirmed (Extended Data Fig. 4a). However, MS177 treatment of liver cancer cells exhibited little EZH2 degradation (Extended Data Fig. 4b,c). We thus designed and synthesized an additional set of EZH2 PROTAC candidates, from which we identified MS7126 as a promising lead. MS7126 utilizes a four-methylene linker to connect the EZH2 inhibitor C24 with the cereblon (CRBN) ligand pomalidomide (Fig. 4a, left). MS7126 is highly selective for EZH2 over other methyltransferases (Extended Data Fig. 4d) and exhibits high-affinity binding to the EZH2 SET domain (Extended Data Fig. 4e). Notably, unlike MS177, MS7126 is efficient at degrading EZH2 in MV4;11 and Huh7 cells (Extended Data Fig. 4a,f), highlighting the context-dependent efficacy of EZH2 PROTACs.

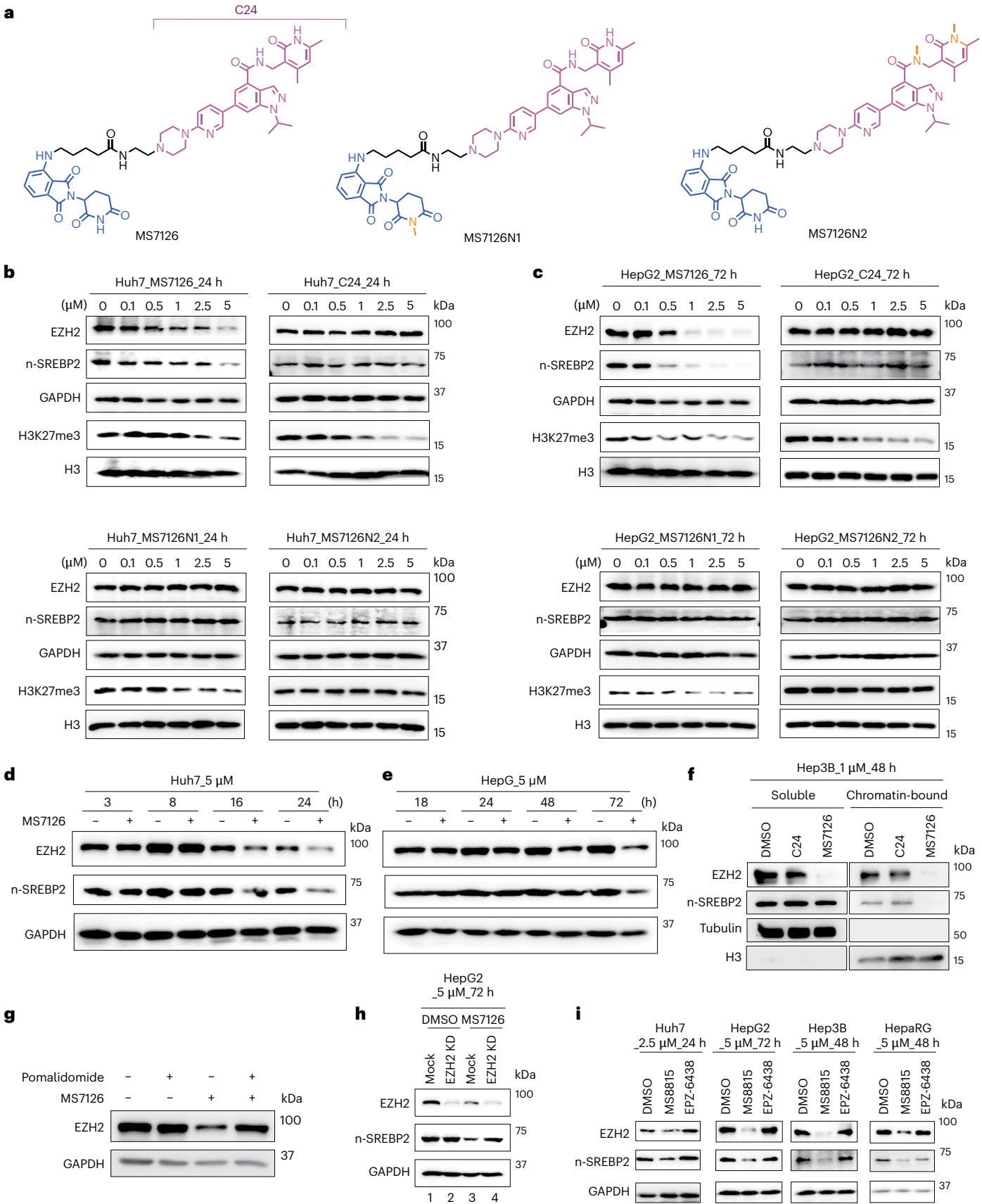
Next, we characterized the effects of MS7126 on-target protein degradation in liver cancer cells. We designed two negative controls: MS7126N1, which contains an additional methyl group in pomalidomide to disrupt CRBN binding (Fig. 4a, middle) and MS7126N2, which has two additional methyl groups in C24 to decrease EZH2 binding, while maintaining CRBN binding (Fig. 4a, right). MS7126 induced EZH2 degradation in a dose-dependent and time-dependent manner across a panel of liver cancer lines, an effect not seen with C24, MS7126N1 or MS7126N2 (Fig. 4b–e and Extended Data Fig. 4g,h). Because MS7126 and MS7126N1 contain an intact C24 moiety (Fig. 4a), they, along with free C24, decreased global H3K27me3, whereas MS7126N2 did not (Fig. 4b,c). Both soluble and chromatin-bound EZH2 were degraded upon treatment with MS7126, but not C24 (Fig. 4f). Notably, EZH2 degradation was blocked by pre-treatment with pomalidomide (Fig. 4g), MLN4924 (a small-molecule inhibitor of the Nedd8-activating enzyme required for assembly of Cullin-based E3 ligase⁴⁵; Extended Data Fig. 4k), or CRBN knockout (KO) (Extended Data Fig. 4l). Collectively, these results demonstrate that MS7126 is a CRBN-dependent EZH2-targeting PROTAC that degrades EZH2 in liver cancer cells.

Given the observed physical association and genome-wide co-localization of EZH2 and n-SREBP2, we next investigated whether MS7126 treatment could also degrade n-SREBP2, a partner of EZH2 bound at EZH2-solo sites, via a bystander effect. The bystander effect occurs when a degrader compound facilitates collateral ubiquitination and degradation of a nearby partner protein⁴⁶. Notably, MS7126 (but not C24, MS7126N1 or MS7126N2) efficiently degraded n-SREBP2 in liver cancer cells as well (Fig. 4b–f). While MS7126 degraded both soluble and chromatin-bound EZH2, it preferentially degraded chromatin-bound n-SREBP2 (Fig. 4f), suggesting a chromatin engagement-dependent EZH2:n-SREBP2 association. Additionally, n-SREBP2-degrading effects of MS7126 strictly depended on the presence of EZH2. MS7126-mediated n-SREBP2 degradation was greatly diminished in *EZH2* KD HepG2 cells (Fig. 4h, lane 4 versus 2 and 3), which contrasted with its robust n-SREBP2 and EZH2 degradation in WT cells (Fig. 4h, lane 3 versus 1). Further, we employed MS8815 (ref. 47), another EZH2-targeting PROTAC developed using a different EZH2 inhibitor (EPZ-6438/tazemetostat) and a different E3 ligand (VHL-recruiting VHL-1). We confirmed the effect of MS8815, but not parent EZH2 inhibitor, on degrading EZH2 and n-SREBP2 in liver cancer cells (Fig. 4i).

Altogether, independent EZH2 PROTACs degrade EZH2 and laterally deplete its functional partner, n-SREBP2, in liver cancer cells.

Fig. 4 | EZH2-targeting PROTAC uniquely degrades both EZH2 and n-SREBP2 in liver cancer cells. **a**, Chemical structures of MS7126, MS7126N1 and MS7126N2. **b,c**, WB of EZH2, n-SREBP2, H3K27me3 and loading controls (GAPDH and total H3) in Huh7 (**b**) and HepG2 cells (**c**) following indicated compound treatment. **d,e**, WB of EZH2, n-SREBP2 and GAPDH in Huh7 (**d**) and HepG2 cells (**e**) treated with 5 μ M MS7126 for the indicated time points. **f**, WB of EZH2 and n-SREBP2 in soluble nucleoplasmic (left) and chromatin-bound (right) fractions of Hep3B cells treated with 1 μ M dimethylsulfoxide (DMSO), C24 or MS7126 for 48 h.

Tubulin and total H3 serve as fractionation controls. **g**, WB of EZH2 and GAPDH in Huh7 cells pre-treated with DMSO or pomalidomide (10 μ M) for 4 h, followed by treatment with DMSO or MS7126 (1 μ M) for 24 h. **h**, WB of EZH2 and n-SREBP2 following indicated treatment with DMSO (lanes 1 and 2) or MS7126 (lanes 3 and 4) in WT (lanes 1 and 3) or *EZH2* KD (lanes 2 and 4) HepG2 cells. **i**, WB of EZH2, n-SREBP2 and GAPDH in Huh7, HepG2, Hep3B and HepaRG cells following the indicated compound treatment. Concentrations and durations are labelled on the top of each panel. Unprocessed blots are available in the Source data file.



Compared with EZH2 enzymatic inhibitors, EZH2 PROTACs exhibit a unique effect on the transcriptome, suppressing cholesterol metabolism in liver cancer cells

To define the impact of EZH2 PROTACs on the transcriptome, we performed RNA-seq in HepG2 cells following treatment with DMSO, C24, MS7126, MS7126N1 or MS7126N2. MS7126 induced robust transcriptomic changes compared with DMSO; in contrast, comparable treatment with C24, MS7126N1 or MS7126N2 caused minimal-to-mild transcriptomic shifts (Fig. 5a, Supplementary Table 4 and Extended Data Fig. 5a–d). Differentially expressed genes (DEGs) that were altered by MS7126 showed a lack of overall changes following treatment with C24 or MS7126N1, whereas MS7126N2 treatment caused only subtle changes (Fig. 5b,c). These results demonstrate that MS7126 is superior to parent enzymatic inhibitor and non-PROTAC analogues in modulating the transcriptome. Notable effects were also observed in Huh7 cells following MS7126 treatment (Extended Data Fig. 5e and Supplementary Table 4). Furthermore, only MS7126 (and not C24, MS7126N1 or MS7126N2) decreased the overall transcription of EZH2-activated genes based on EZH2 KD studies (Extended Data Fig. 5f). As expected, GSEA detected a positive correlation between MS7126 treatment and the reactivation of PRC2-targeted genes in both models (Extended Data Fig. 5g,h). Additionally, RT–qPCR verified MS7126's robust gene-derepressing effects on select PRC2:H3K27me3 targets (Extended Data Fig. 5i,j).

Consistent with genetic *EZH2* depletion (Fig. 2a,b), GSEA revealed that cholesterol homeostasis and fatty acid metabolism genes were among the top-ranked downregulated pathways following MS7126 treatment in HepG2 and Huh7 cells (Fig. 5d,e and Supplementary Table 4). A more detailed GSEA of SREBP2- and MVA pathway-related gene sets also confirmed their downregulation by MS7126 (Fig. 5f,g and Extended Data Fig. 5k,l), mirroring genetic *EZH2* depletion (Fig. 2c,d). In contrast, these correlations were not observed following treatment with C24, MS7126N1 or MS7126N2. RT–qPCR further validated that MS7126 (but not C24, MS7126N1 or MS7126N2) robustly and consistently repressed MVA pathway genes in HepG2 (Fig. 5h) and Huh7 cells (Fig. 5i). As lipid deprivation is known to activate SREBP2 (refs. 19,48), we employed a culture medium supplemented with delipidated serum. We first confirmed that this medium activates MVA pathway genes in control cells and further found that MS7126 robustly downregulated these MVA pathway genes under delipidated conditions, whereas C24, MS7126N1 and MS7126N2 could not (Extended Data Fig. 5m). Furthermore, a cross-comparison of DEGs downregulated following both genetic depletion and pharmacological targeting of EZH2 defined a 26-gene signature in Huh7 and

HepG2 cells (Fig. 5j). Again, this EZH2-activated transcript signature is notably enriched for cholesterol metabolism genes and known SREBP2 targets (Fig. 5k), consistent with EZH2 cistrome studies (Figs. 1h and 2h,i). An independent EZH2 PROTAC (MS8815, comprising EPZ-6438 and a VHL-recruiting ligand), but not its matched inactive analogue (MS8815N), also diminished MVA pathway genes in Huh7 and HepG2 cells (Fig. 5l,m). In contrast, an EED-targeting PROTAC⁴⁹ did not alter expression of these genes (Extended Data Fig. 5n,o), mirroring genetic EED depletion (Extended Data Fig. 2q,r). These data again support a PRC2(EED)-independent function of EZH2. Consistent with MVA pathway gene downregulation, MS7126 treatment consistently decreased cellular cholesterol concentrations compared with treatment with DMSO, C24, MS7126N1, MS7126N2 or pomalidomide in Huh7 (Fig. 5n) and HepG2 cells (Extended Data Fig. 5p).

Collectively, EZH2 is positively associated with increased transcription of MVA pathway genes and elevated cellular cholesterol production in liver cancer cells. Compared with non-degrading analogues and the EED degrader, independent EZH2 PROTACs exert unique and superior inhibitory effects on cholesterol metabolism.

EZH2 PROTACs exhibit superior tumour-suppressing effects compared with EZH2 enzymatic inhibitors in liver cancer

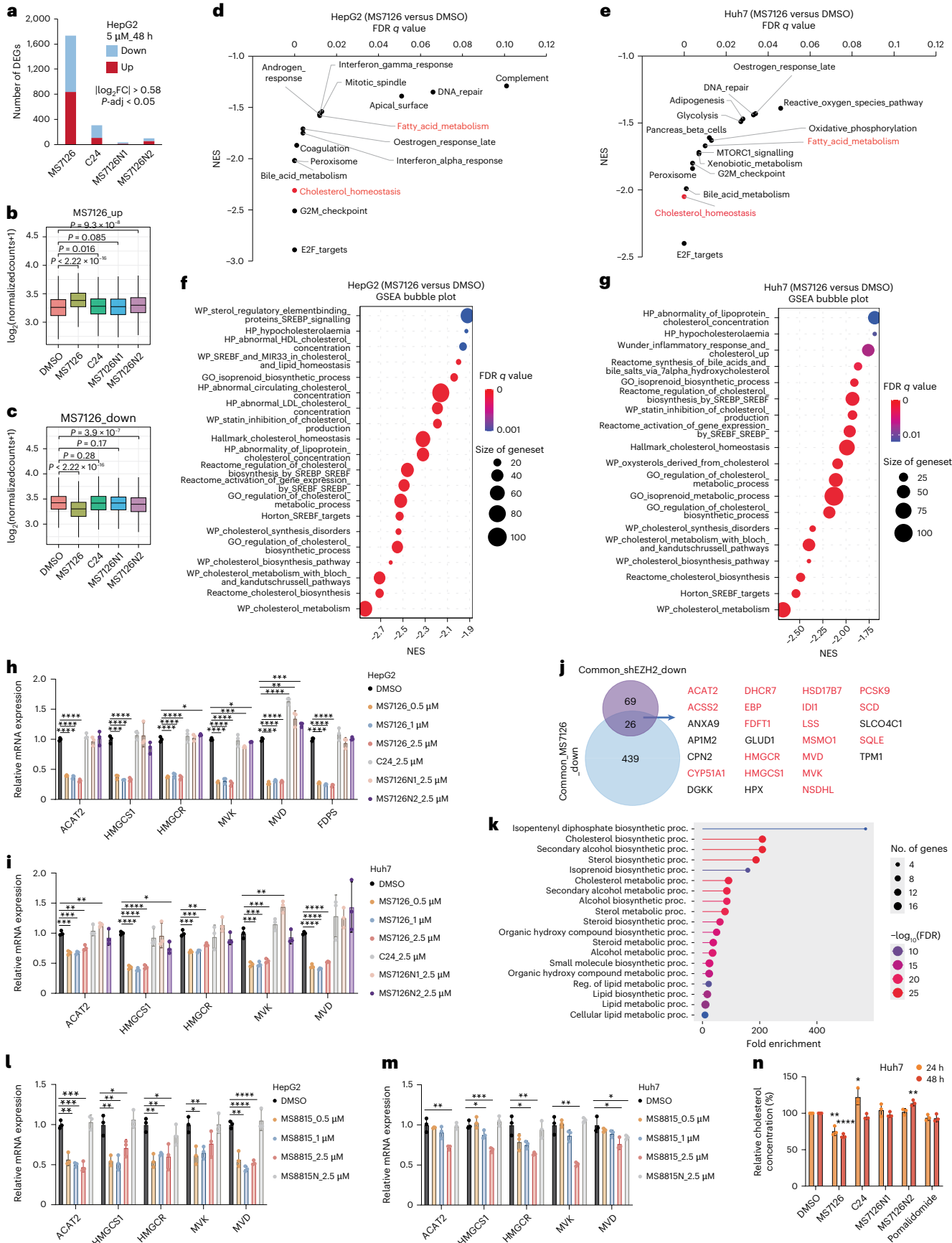
To assess the anti-tumour effects of EZH2 PROTACs, we used MS7126 and matched control compounds to assay proliferation across a panel of human liver cancer lines (Supplementary Table 5 and Extended Data Fig. 6a–d). MS7126 exhibited a strong inhibitory effect in vitro, with half-maximal effective concentration (EC_{50}) values ranging from 28 to 832 nM following a 6-day treatment (Supplementary Table 5). In contrast, treatments with C24, MS7126N1 or MS7126N2 showed little-to-mild effects (Supplementary Table 5 and Extended Data Fig. 6a–d). Furthermore, MS7126 and C24 exhibited minimal toxicity in non-tumorigenic liver epithelial cells (Supplementary Table 5 and Extended Data Fig. 6e). Colony-forming assays consistently detected that MS7126 and MS8815 achieved superior growth inhibition compared with their respective controls across multiple models, including human cancer lines and an aggressive murine liver tumour line carrying *Nras* mutation and *p53* loss (NSP)⁵⁰ (Fig. 6a–f and Extended Data Fig. 6f–i). As an additional control, treatment with pomalidomide did not inhibit Huh7 cell proliferation (Fig. 6a,b).

Having observed promising anti-tumour effects in vitro, we next characterized the pharmacokinetic (PK) properties of MS7126 in mice. Administration of a single 25 mg kg^{−1} dose of MS7126 achieved plasma concentrations comparable with the EC_{50} value measured in our in vitro studies (Fig. 6g and Supplementary Table 6). Subsequently,

Fig. 5 | Compared with the matched non-degrader compound and EED PROTAC, EZH2 PROTAC exhibits superior and unique effects on suppressing MVA pathway genes and cholesterol metabolism in liver cancer cells.

a, Bar chart showing the total number of DEGs in HepG2 cells following a 48-h treatment with 5 μ M MS7126, C24, MS7126N1 or MS7126N2, relative to DMSO, identified by RNA-seq ($n = 2$ replicated experiments). **b,c**, Box plots displaying overall expression levels across indicated cell treatment groups for upregulated (**b**) or downregulated (**c**) DEGs identified in MS7126- versus DMSO-treated HepG2 cells, based on RNA-seq ($n = 2$ replicated experiments). Box boundaries indicate the 25th–75th percentiles, centre lines indicate median and whiskers indicate 1.5 $\times$ interquartile range. A two-sided Wilcoxon rank-sum test was used for P value calculations. **d,e**, GSEA summary plots showing the negative enrichment of the indicated hallmark pathways in HepG2 (**d**) or Huh7 (**e**) cells following MS7126 treatment versus DMSO. **f,g**, Bubble plots showing the indicated gene set enrichment based on GSEA and RNA-seq profiles of HepG2 (**f**) or Huh7 (**g**) cells following MS7126 treatment versus DMSO. Bubble colour and size represent the FDR q value and gene set size, respectively; the x axis shows NES. **h,i**, RT–qPCR for MVA pathway genes in HepG2 (**h**; 48 h) and Huh7 cells (**i**; 24 h) treated with the indicated concentrations of MS7126, C24, MS7126N1 or MS7126N2, relative to DMSO. The y axis shows the mean $\pm$ s.d. of relative transcription levels

normalized to an internal control and DMSO-treated samples ($n = 3$ independent experiments). P values were calculated by an unpaired, two-tailed Student's t -test ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$). **j,k**, Venn diagram (**j**) showing overlapping downregulated DEGs in both Huh7 and HepG2 cells following *EZH2* KD (**j**, top) or MS7126 treatment (**j**, bottom) to identify a 26-gene EZH2-activated target signature (cholesterol metabolism genes highlighted in red in **j**, right), followed by GO analysis (**k**). **l,m**, RT–qPCR for MVA pathway genes in HepG2 (**l**; 48 h) and Huh7 cells (**m**; 24 h) treated with the indicated concentrations of MS8815 or MS8815N, relative to DMSO. The y axis shows mean $\pm$ s.d. of relative transcription levels normalized to an internal control and DMSO-treated samples ($n = 3$ independent experiments). P values were calculated by an unpaired, two-tailed Student's t -test ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$). **n**, Measurement of cholesterol concentration in Huh7 cells following treatment for the indicated durations with DMSO, MS7126, C24, MS7126N1, MS7126N2 or pomalidomide (5 μ M). The y axis shows mean $\pm$ s.d. of relative cholesterol concentrations normalized to DMSO-treated samples ($n = 3$ independent experiments). P values were calculated by an unpaired, two-tailed Student's t -test ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.0001$). Source numerical data and P values are available in the Source data file.



we established human cancer line-derived xenograft (CDX) models with Huh7 and HepG2 cells to evaluate the in vivo efficacy of MS7126 versus C24. MS7126 (25 mg kg⁻¹, twice per day) significantly suppressed tumour development compared with vehicle controls in both HepG2 (Fig. 6h) and Huh7-derived CDXs (Fig. 6i). A comparable dose of C24 (based on previous studies¹⁷) showed no effect on tumour progression in vivo (Fig. 6h,i). Furthermore, we observed a significant decrease in the expression of MVA pathway genes in tumours isolated from MS7126-treated mice (Fig. 6j). Moreover, we assessed MS8815, either singly or in combination with doxorubicin, in the aggressive mouse liver tumour model, NSP⁵⁰. Treatment with MS8815 alone significantly delayed tumour growth in a dose-dependent manner, whereas combined dosing with doxorubicin further improved anti-tumour efficacy relative to monotherapy (Fig. 6k). Of note, the lack of significant animal weight loss supports the tolerability of EZH2 PROTACs for in vivo dosing (Extended Data Fig. 6j,k and Fig. 6l).

Collectively, EZH2-targeting PROTACs represent promising anti-tumour agents for liver cancer, as supported by both in vitro and in vivo evidence.

EZH2-dependent upregulation of cholesterol biosynthesis genes extends to diverse cancers

Having defined the roles of EZH2 and n-SREBP2 in upregulating MVA pathway genes and demonstrated the efficacy of EZH2 PROTACs, we next queried whether this axis exists in other malignancies. Using publicly available datasets, we observed a general correlation between *EZH2* and *SREBP2* transcription across cancer cell lines and TCGA samples (Fig. 7a,b and Extended Data Fig. 7). For example, diffuse large B cell lymphoma (DLBCL), which is a cancer type typically believed to be driven by EZH2:PRC2 hyperactivity^{9,21}, exhibited a high positive correlation between *EZH2* and *SREBP2* expression in TCGA DLBCL samples (Fig. 7b), where the two also show elevated levels compared with healthy tissues (Extended Data Fig. 8a,b). These observations indicate a synergy between EZH2 and SREBP2 in promoting aggressive phenotypes beyond liver cancer. To test this idea, we conducted cleavage under targets and tagmentation (CUT&Tag) for EZH2, H3K27me3, H3K27ac and n-SREBP2 in WEHI-231 B cell lymphoma cells. Cross-comparison between EZH2 and H3K27me3 peaks showed that EZH2-solo peaks account for the vast majority of EZH2 peaks; these peaks lack H3K27me3 and are enriched for H3K27ac (Fig. 7c, top). Unbiased motif search further revealed enrichment for the n-SREBP2 consensus motif at EZH2-solo peaks (Fig. 7d). Subsequently, we verified the co-localization of EZH2-solo binding with n-SREBP2 and H3K27ac in WEHI-231 cells (Fig. 7c,e). Classic MVA pathway genes are co-bound by EZH2, n-SREBP2 and H3K27ac (but not H3K27me3) in this lymphoma model (Fig. 7f and Extended Data Fig. 8c).

We next assessed the ability of EZH2 PROTACs to degrade EZH2 and n-SREBP2 in lymphoma. Compared with EPZ-6438, MS8815

readily induced degradation of both EZH2 and n-SREBP2 in human DLBCL lines (SU-DHL-6 and OCI-LY1) and murine B cell lymphoma lines (WEHI-231 and A20) (Fig. 7g). Furthermore, RNA-seq revealed that MS8815 caused a profound transcriptomic perturbation; in contrast, comparable EPZ-6438 or MS8815N treatment had a minimal impact, with only a few genes showing differential expression (Extended Data Fig. 8d–g and Supplementary Tables 7 and 8). Of note, GSEA revealed that MS8815 treatment was associated with the downregulation of transcripts related to cholesterol biosynthesis and n-SREBP2 targets in WEHI-231 and SU-DHL-6 cells (Fig. 7h,i and Supplementary Tables 7 and 8). RT-qPCR validated the unique effect by MS8815 and not EPZ-6438 or MS8815N, on suppressing MVA pathway genes (Fig. 7j,k). Compared with these matched controls, MS8815 also exhibited superior cytotoxicity in B cell lymphoma models (Extended Data Fig. 8h).

Additionally, we assessed the EZH2–n-SREBP2–MVA axis using 22Rv1 cells, an AR-positive prostate cancer line, and MV4;11 cells, an MLL-r AML line. Genome-wide mapping in both models uncovered that the majority of EZH2-binding sites lack H3K27me3 and exhibit co-binding of EZH2-solo, H3K27ac and n-SREBP2 (Fig. 8a–c and Extended Data Fig. 9a–c). Consistent with our findings in liver cancer and B cell lymphoma, MVA pathway genes in these cells are co-bound by EZH2, n-SREBP2 and H3K27ac but not H3K27me3 (Fig. 8d and Extended Data Fig. 9d), contrasting with PRC2 targets that are marked by EZH2 and H3K27me3 but lack H3K27ac and n-SREBP2 binding (Extended Data Fig. 9e). RNA-seq and subsequent RT-qPCR detected that MS177 uniquely downregulated SREBP2 targets and cholesterol biosynthesis genes in both 22Rv1 and MV4;11 cells (Fig. 8e,f and Extended Data Fig. 9f,g). Consistently, treatment with EZH2 PROTACs (MS8815 or MS177), and not matched enzymatic inhibitors (EPZ-6438 or C24), degraded EZH2 and n-SREBP2 in AR-positive prostate cancer cells (LNCaP and 22Rv1; Fig. 8g) and MV4;11 AML cells (Extended Data Fig. 9h).

Together, these exist a previously unappreciated role for EZH2 in enhancing the MVA pathway and cholesterol metabolism across diverse cancers. EZH2-targeting degraders offer a promising strategy to co-deplete n-SREBP2 and treat tumours dependent on the EZH2–n-SREBP2–MVA axis (Fig. 8h).

Discussion

Cholesterol metabolism represents a crucial therapeutic target in cancer²⁹. Enhanced cholesterol biosynthesis and lipid metabolism promote tumorigenesis by fuelling cell proliferation, maintaining stemness, producing treatment-resistant phenotypes^{51–53}, modulating ferroptosis^{54,55} and suppressing anti-tumour immunity^{29,56,57}. Previous works suggested a linkage between EZH2 and lipid metabolism⁵⁸. For example, a CRISPR screen recently identified the cholesterol biosynthesis pathway as a targetable vulnerability in germinal centre B cell-like (GCB) DLBCL

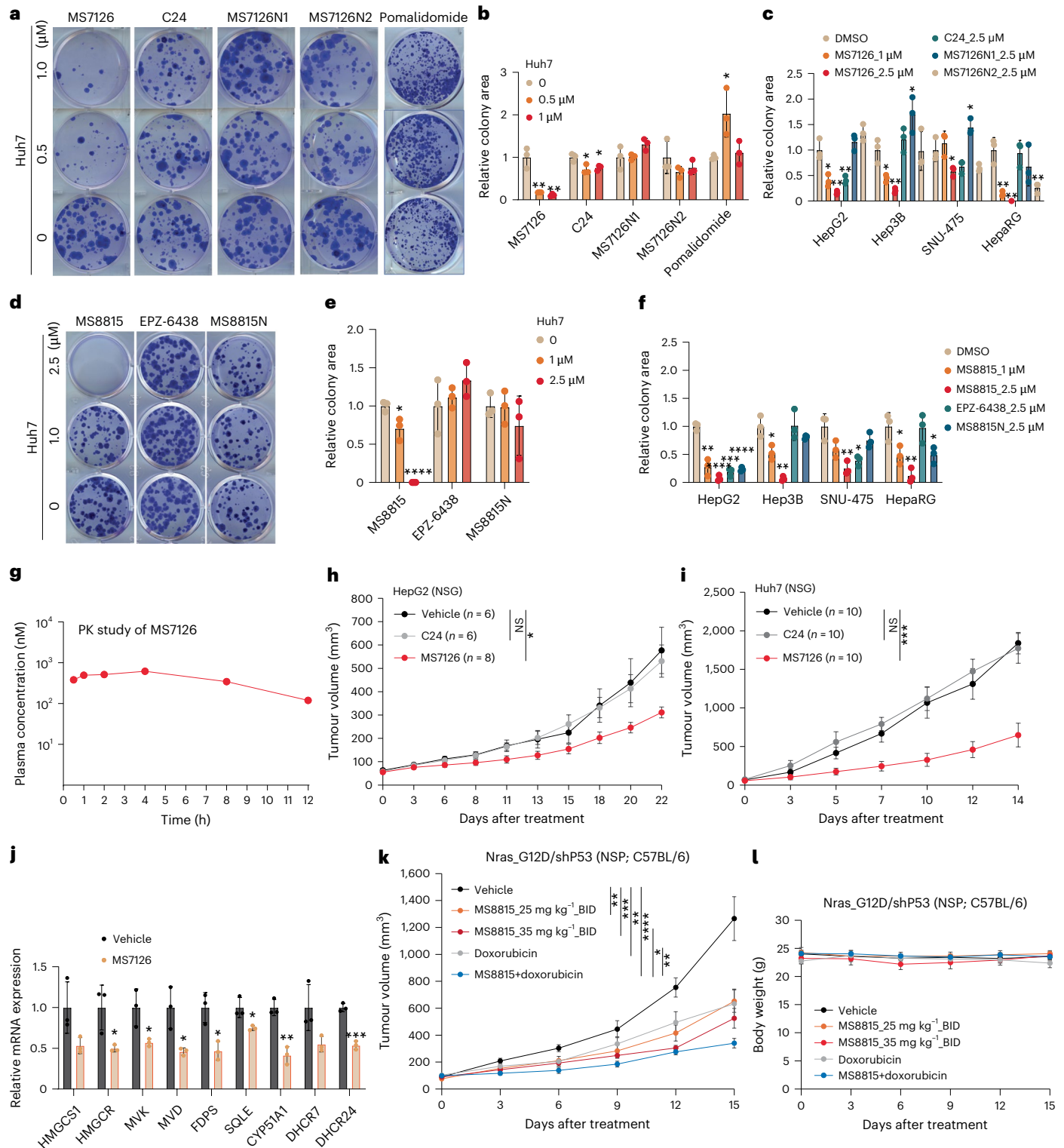
Fig. 6 | EZH2-targeting PROTACs exhibit superior tumour-suppressing effects in vitro and in vivo compared with parent EZH2 enzymatic inhibitors.

a–f, Representative images (**a,d**) and quantification (**b,c,e,f**) of colony formation by liver cancer lines, treated with the indicated concentrations of compounds. The y axis in **b, c, e, f** shows the mean $\pm$ s.d. of colony area relative to mock-treated controls ($n = 3$ independent experiments). P value was calculated by an unpaired, two-tailed Student's t -test (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$). **g**, Plasma concentration of MS7126 following a single intraperitoneal (i.p.) administration to male Swiss albino mice ($n = 3$ per time point). **h, i**, Growth of HepG2 (**h**) and Huh7 cell line-derived (i) xenograft (CDX) tumours in vivo. Cells were subcutaneously transplanted into NSG mice, followed by in vivo dosing with either vehicle, 10 mg kg⁻¹ C24 or 25 mg kg⁻¹ MS7126. Each compound was administered via i.p. injection twice per day (BID) for 5 consecutive days per week (n , the number of mice). Data show the CDX tumour size as mean $\pm$ s.e.m. P values were calculated by a two-way analysis of variance (ANOVA) or mixed effects analysis (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). **j**, RT-qPCR for MVA pathway genes in

HepG2-derived CDX tumours isolated from mice treated with vehicle or MS7126. The y axis shows the mean $\pm$ s.e.m. of relative transcription levels normalized to an internal control and vehicle-treated samples ($n = 3$ independent experiments). P values were calculated by an unpaired, two-tailed Student's t -test (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). **k, l**, In vivo growth of allografted tumours (**k**) and body weight measurements of mice (**l**) bearing murine liver tumours that express *Nras*, *GLI2D* and *shP53* ('NSP'). 'NSP' cells were subcutaneously transplanted into syngeneic C57BL/6 mice, followed by dosing with vehicle ($n = 14$), 25 mg kg⁻¹ MS8815 ($n = 8$), 35 mg kg⁻¹ of MS8815 ($n = 8$), 1 mg kg⁻¹ doxorubicin ($n = 8$) or a combination of 35 mg kg⁻¹ MS8815 and 1 mg kg⁻¹ doxorubicin ($n = 8$). MS8815 was administered via i.p. (BID) with occasional drug holidays and doxorubicin was administered i.p. once per week for a total of two injections. The y axis shows the tumour size as mean $\pm$ s.e.m. P values were calculated by a two-way ANOVA (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$). Source numerical data and P values are available in the Source data file.

exhibiting EZH2 inhibitor resistance⁵⁹. In HCC, an off-target effect of the EZH2 enzymatic inhibitor GSK343 was reported to activate SREBP2, which can be suppressed by combinational treatment with the site-1 protease (S1P) inhibitor PF-429242 (ref. 60). Our studies not only provide a molecular explanation for the linkage between EZH2 and cholesterol biosynthesis but offer a potent monotherapy strategy by utilizing EZH2 PROTACs to simultaneously co-degrade EZH2 and n-SREBP2. Because n-SREBP2 TF lacks defined druggable domains, targeting it directly remains a challenge. Inhibitors such as fatostatin⁶¹ and PF-429242 were developed to target the cytosolic SREBP2 processing

via blocking its ER-to-Golgi translocation or S1P-mediated proteolytic cleavage. Fatostatin suppressed MVA pathway gene expression and proliferation of liver cancer cells (Extended Data Fig. 9i–k), mirroring *SREBP2* depletion. Given that MS7126 exerts a direct degradation effect on chromatin-bound n-SREBP2 (Fig. 4f), the combined usage of fatostatin and EZH2 PROTACs elicited stronger tumour-inhibitory effects (Extended Data Fig. 9k), suggesting a more complete inhibition of SREBP2 via simultaneously targeting cytosolic SREBP2 maturation and n-SREBP2. Aside from repressing MVA pathway genes, EZH2 PROTACs derepress PRC2:H3K27me3-targeted TSGs, leading to improved



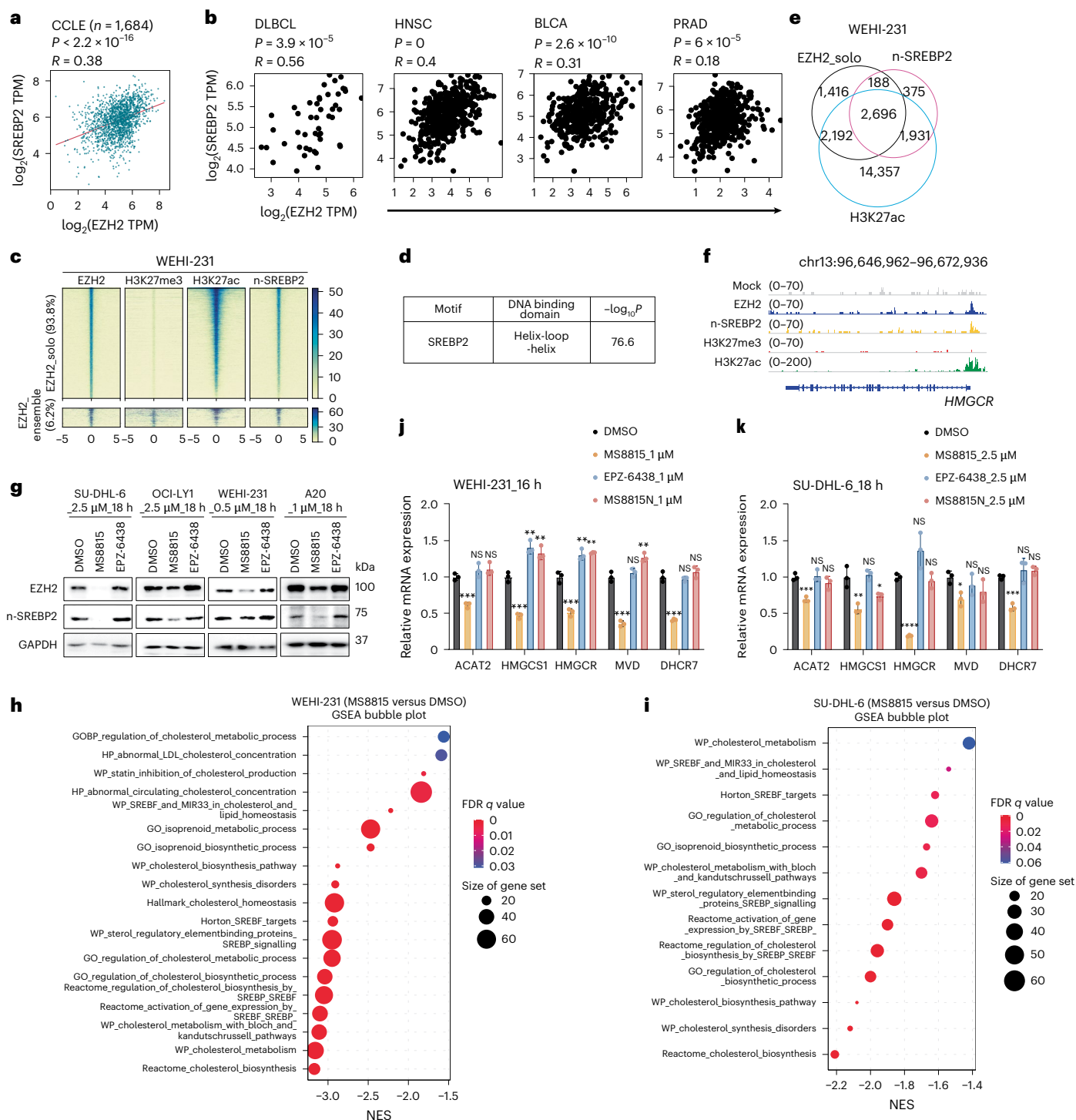


Fig. 7 | EZH2 potentiates the expression of MVA pathway genes in B cell lymphoma. a, b, Scatter-plots showing the relationship between *EZH2* and *SREBP2* expression levels among 1,684 human cancer cell lines in the DepMap Public_25Q2 dataset (**a**) and in primary tumours of the indicated TCGA patient cohorts (**b**), such as DLBCL, head and neck squamous cell carcinoma (HNSC), bladder urothelial carcinoma (BLCA) and prostate adenocarcinoma (PRAD). Each dot represents an individual cell line or tumour sample, with $\log_2$ -transformed transcripts per million (TPM) for *EZH2* and *SREBP2* shown on the x axis and y axis, respectively. The Pearson correlation coefficient (R) and P value are displayed at the top of each panel. **c**, Unsupervised clustering using CUT&Tag peaks of *EZH2*, H3K27me3, H3K27ac and n-SREBP2, ± 5 kb from *EZH2* peak centres in the WEHI-231 cells. **d**, Enrichment of the SREBP2 consensus motif at *EZH2*-solo binding sites in WEHI-231 cells, determined via the SeqPos tool. **e**, Venn diagram showing the overlap among *EZH2*-solo, n-SREBP2 and H3K27ac peaks in the WEHI-231

B cell lymphoma cells. **f**, IGV views showing CUT&Tag signals of the indicated proteins at *HMGCR* in WEHI-231 cells. **g**, WB for *EZH2* and n-SREBP2 following the indicated compound treatment of B cell lymphoma cells. **h, i**, Bubble plots showing indicated gene set enrichment based on GSEA and RNA-seq profiles of WEHI-231 (**h**) and SU-DHL-6 cells (**i**) following treatment with MS8815 compared with DMSO. Bubble colour and size represent the FDR q value and gene set size, respectively; the x axis shows NES. **j, k**, RT-qPCR for MVA pathway genes in WEHI-231 (**j**) and SU-DHL-6 cells (**k**) following the indicated compound treatments. The y axis shows the mean $\pm$ s.d. of relative transcription levels normalized to an internal control and DMSO-treated samples ($n = 3$ independent experiments). P values were calculated by an unpaired, two-tailed Student's t -test ($*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$). Unprocessed blots, source numerical data and P values are available in the Source data file.

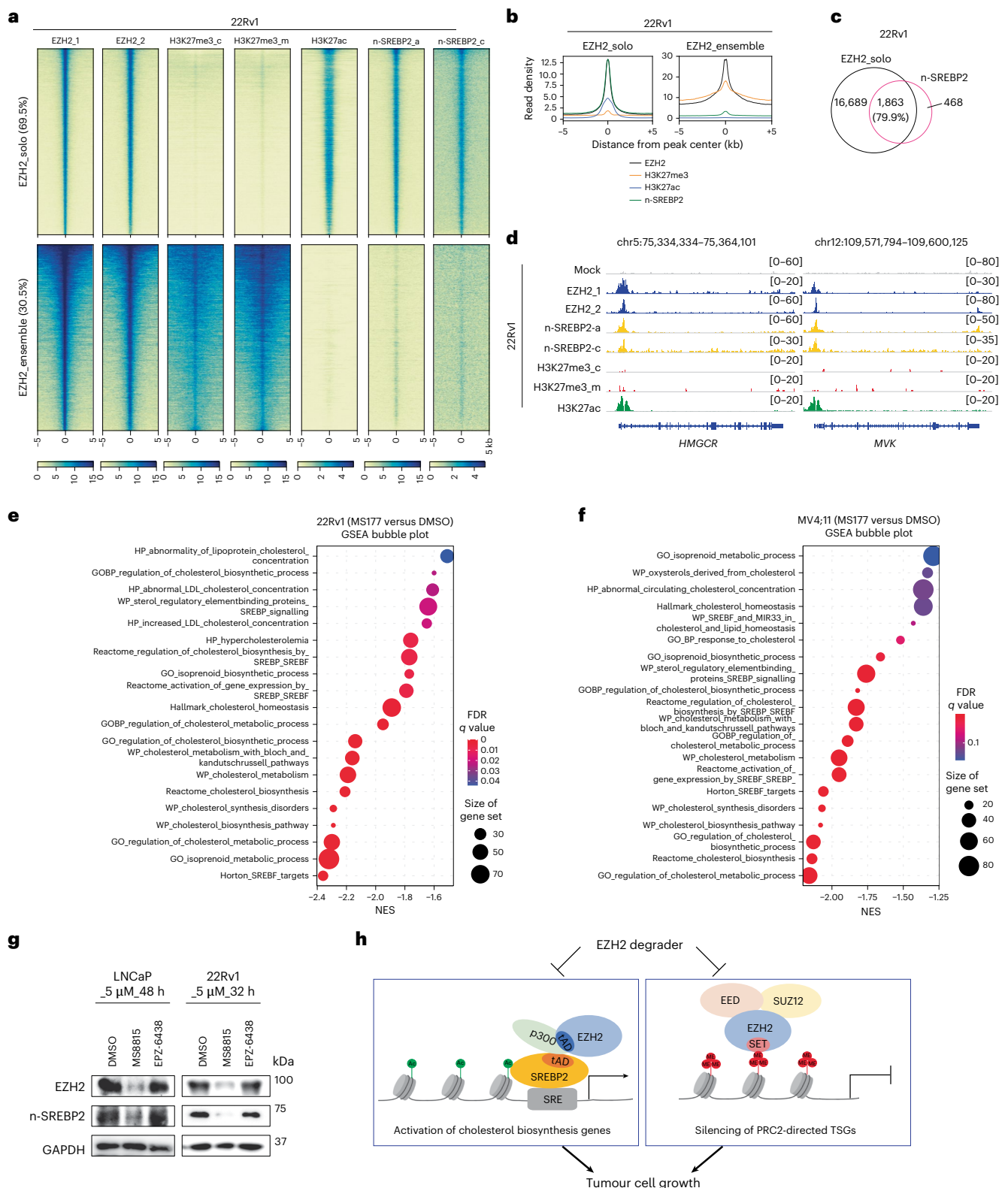


Fig. 8 | EZH2–n-SREBP2–MVA pathway is present in prostate cancer and MLL-r leukaemia. a, b, Heatmaps (**a**) and averaged signal intensities (**b**) of peaks for EZH2, H3K27me3, n-SREBP2 and H3K27ac, ± 5 kb from EZH2 peak centres in 22Rv1 cells. **c,** Venn diagram displaying the overlap between EZH2-solo and n-SREBP2 peaks in 22Rv1 cells. **d,** IGV views showing genomic signal enrichment of the indicated proteins at *HMGR* and *MVK* in 22Rv1 cells. **e, f,** Bubble plots showing indicated gene set enrichment based on GSEA and RNA-seq profiles of 22Rv1 (**e**) and MV4;11 cells (**f**) following treatment with MS177 versus DMSO. Bubble colour

and size represent the FDR q value and gene set size, respectively; the x axis shows NES. **g,** WB for EZH2 and n-SREBP2 following the indicated compound treatments of LNCaP and 22Rv1 cells. **h,** Schematic model illustrating that EZH2:n-SREBP2:p300-dependent activation of MVA pathway genes and the cholesterol biosynthesis programme (left), along with EZH2:PRC2:H3K27me3-dependent silencing of TSGs (right), collectively establish cancer-promoting epigenomic, transcriptomic and metabolic programmes, to drive the development of aggressive cancers. Unprocessed blots are available in the Source data file.

tumour inhibition (Fig. 8h). Of note, EZH2 and SREBP2 have also been implicated in cisplatin resistance in ovarian cancer⁶². Further investigation is required to assess the anti-cancer activity of EZH2 PROTACs in different settings.

The mechanisms governing the switch between EZH2's canonical repressive role and non-canonical activating role warrant investigation. Previous reports point to dysregulated kinase signalling in cancer. AKT, CDK1 and JAK3 induce site-specific phosphorylation of EZH2, which reduces its affinity for histone H3, promotes its dissociation from PRC2 (refs. 15,16,63) and may trigger a conformational change that unlocks cryptic tADs, resulting in enhanced binding to p300 (refs. 36,37). Conversely, the loss of PKC α /i in advanced prostate cancer stabilizes PRC2-free EZH2, prompting a switch to a non-canonical EZH2:YY1-dependent cistrome that activates translation machinery and confers drug resistance¹³. Interplays exist between EZH2 and RNAs, which may drive EZH2's functional shift^{64–67}. Additionally, EZH2-elicited gene-activation effects are complex and can be achieved through both methyltransferase-dependent^{13,68} and methyltransferase-independent mechanisms^{14,15,36,37}. Conceivably, a collection of potential factors (such as dysregulated kinases, increased EZH2, p300 and/or onco-TF levels and EZH2–RNA interactions) may facilitate the transition of EZH2 from a corepressor to a co-activator during malignant transformation. Additional research effort shall be directed towards dissecting multivalent interactions among EZH2, p300 and onco-TFs. A deeper understanding of this switch is essential for developing effective therapeutic strategies.

Using parent inhibitors and PROTAC-inactive analogues, we demonstrated the unique effects of independent EZH2 PROTACs (MS7126 and MS8815) on degrading not only the primary target (EZH2) but also its functional partner (n-SREBP2) across multiple tumours. The n-SREBP2-degrading effect of EZH2 PROTACs relies on the presence of EZH2 (Fig. 4h), pointing to an on-target bystander effect. Similar bystander effects were observed with other degraders. For instance, the EED-targeting PROTAC #2 degrades EED along with EZH2 and SUZ12 (ref. 49). Another EED-targeting PROTAC, MS147, degrades both the PRC2 and PRC1 complexes⁶⁹. Notably, a CDK4/6-targeting PROTAC, MS28, degrades the CDK4/6-associated cyclin D1 with faster kinetics than the primary target, CDK4/6 itself⁷⁰. Notably, certain PROTACs may not be effective across all cancer models, likely due to varying cellular contexts (for example, the expression level and availability of the recruited E3 ligase, or the stability and permeability of the compound in different cell types). While further optimization of EZH2-targeting PROTACs is required to improve their potency, we remain optimistic that these degraders will provide a promising therapeutic strategy for cancers dependent on EZH2 and/or MVA pathway dysregulation.

Online content

Any methods, additional references, Nature Portfolio 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/s41556-026-02048-x>.

References

- Hanahan, D. Hallmarks of cancer: new dimensions. *Cancer Discov.* **12**, 31–46 (2022).
- Sabari, B. R., Zhang, D., Allis, C. D. & Zhao, Y. Metabolic regulation of gene expression through histone acylations. *Nat. Rev. Mol. Cell Biol.* **18**, 90–101 (2017).
- Badeaux, A. I. & Shi, Y. Emerging roles for chromatin as a signal integration and storage platform. *Nat. Rev. Mol. Cell Biol.* **14**, 211–224 (2013).
- Margueron, R. & Reinberg, D. The Polycomb complex PRC2 and its mark in life. *Nature* **469**, 343–349 (2011).
- Guo, Y., Zhao, S. & Wang, G. G. Polycomb gene silencing mechanisms: PRC2 chromatin targeting, H3K27me3 'readout', and phase separation-based compaction. *Trends Genet.* **37**, 547–565 (2021).
- Kim, K. H. & Roberts, C. W. Targeting EZH2 in cancer. *Nat. Med.* **22**, 128–134 (2016).
- Varambally, S. et al. The Polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature* **419**, 624–629 (2002).
- Wu, S. Y. et al. The correlation of EZH2 expression with the progression and prognosis of hepatocellular carcinoma. *BMC Immunol.* **23**, 28 (2022).
- Xu, B., Konze, K. D., Jin, J. & Wang, G. G. Targeting EZH2 and PRC2 dependence as novel anticancer therapy. *Exp. Hematol.* **43**, 698–712 (2015).
- Kang, Y., Zhang, Y. & Sun, Y. Comprehensive analysis of the expression characteristics of the enhancer of the zeste homolog 2 gene in pan-cancer. *Front. Genet.* **12**, 658241 (2021).
- Xu, K. et al. EZH2 oncogenic activity in castration-resistant prostate cancer cells is Polycomb-independent. *Science* **338**, 1465–1469 (2012).
- Venkadakrishnan, V. B. et al. Lineage-specific canonical and non-canonical activity of EZH2 in advanced prostate cancer subtypes. *Nat. Commun.* **15**, 6779 (2024).
- Chatterjee, S. S. et al. Increased translation driven by non-canonical EZH2 creates a synthetic vulnerability in enzalutamide-resistant prostate cancer. *Nat. Commun.* **15**, 9755 (2024).
- Lee, S. T. et al. Context-specific regulation of NF- κ B target gene expression by EZH2 in breast cancers. *Mol. Cell* **43**, 798–810 (2011).
- Wei, Y. et al. CDK1-dependent phosphorylation of EZH2 suppresses methylation of H3K27 and promotes osteogenic differentiation of human mesenchymal stem cells. *Nat. Cell Biol.* **13**, 87–94 (2011).
- Yan, J. et al. EZH2 phosphorylation by JAK3 mediates a switch to noncanonical function in natural killer/T-cell lymphoma. *Blood* **128**, 948–958 (2016).
- Wang, J. et al. EZH2 noncanonically binds cMyc and p300 through a cryptic transactivation domain to mediate gene activation and promote oncogenesis. *Nat. Cell Biol.* **24**, 384–399 (2022).
- Wang, L. et al. EZH2 depletion potentiates MYC degradation inhibiting neuroblastoma and small cell carcinoma tumor formation. *Nat. Commun.* **13**, 12 (2022).
- Brown, M. S. & Goldstein, J. L. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell* **89**, 331–340 (1997).
- Mullen, P. J., Yu, R., Longo, J., Archer, M. C. & Penn, L. Z. The interplay between cell signalling and the mevalonate pathway in cancer. *Nat. Rev. Cancer* **16**, 718–731 (2016).
- Yap, D. B. et al. Somatic mutations at EZH2 Y641 act dominantly through a mechanism of selectively altered PRC2 catalytic activity, to increase H3K27 trimethylation. *Blood* **117**, 2451–2459 (2011).
- Dale, B. et al. Advancing targeted protein degradation for cancer therapy. *Nat. Rev. Cancer* **21**, 638–654 (2021).
- Rumgay, H. et al. Global burden of primary liver cancer in 2020 and predictions to 2040. *J. Hepatol.* **77**, 1598–1606 (2022).
- Arzumanyan, V. A., Kiseleva, O. I. & Poverennaya, E. V. The curious case of the HepG2 cell line: 40 years of expertise. *Int. J. Mol. Sci.* **22**, 13135 (2021).
- Horton, J. D., Goldstein, J. L. & Brown, M. S. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J. Clin. Invest.* **109**, 1125–1131 (2002).

26. Seo, Y. K. et al. Genome-wide localization of SREBP-2 in hepatic chromatin predicts a role in autophagy. *Cell Metab.* **13**, 367–375 (2011).
27. Valenza, M., Birolini, G. & Cattaneo, E. The translational potential of cholesterol-based therapies for neurological disease. *Nat. Rev. Neurol.* **19**, 583–598 (2023).
28. Duan, Y. et al. Regulation of cholesterol homeostasis in health and diseases: from mechanisms to targeted therapeutics. *Signal Transduct. Target Ther.* **7**, 265 (2022).
29. Huang, B., Song, B. L. & Xu, C. Cholesterol metabolism in cancer: mechanisms and therapeutic opportunities. *Nat. Metab.* **2**, 132–141 (2020).
30. Juarez, D. & Fruman, D. A. Targeting the mevalonate pathway in cancer. *Trends Cancer* **7**, 525–540 (2021).
31. Schug, Z. T. et al. Acetyl-CoA synthetase 2 promotes acetate utilization and maintains cancer cell growth under metabolic stress. *Cancer Cell* **27**, 57–71 (2015).
32. Mesmin, B. et al. STARD4 abundance regulates sterol transport and sensing. *Mol. Biol. Cell* **22**, 4004–4015 (2011).
33. Paton, C. M. & Ntambi, J. M. Biochemical and physiological function of stearoyl-CoA desaturase. *Am. J. Physiol. Endocrinol. Metab.* **297**, E28–E37 (2009).
34. Hu, A. et al. PIP4K2A regulates intracellular cholesterol transport through modulating PI(4,5)P(2) homeostasis. *J. Lipid Res.* **59**, 507–514 (2018).
35. Wang, S. et al. Target analysis by integration of transcriptome and ChIP-seq data with BETA. *Nat. Protoc.* **8**, 2502–2515 (2013).
36. Jiao, L. et al. A partially disordered region connects gene repression and activation functions of EZH2. *PNAS* **117**, 16992–17002 (2020).
37. Becht, D. C. et al. Multiple transactivation domains of EZH2 bind to the TAZ2 domain of p300 and stimulate the acetyltransferase function of p300. *Biochem. J.* **482**, 955–968 (2025).
38. Giandomenico, V., Simonsson, M., Gronroos, E. & Ericsson, J. Coactivator-dependent acetylation stabilizes members of the SREBP family of transcription factors. *Mol. Cell. Biol.* **23**, 2587–2599 (2003).
39. Janicki, S. M. et al. From silencing to gene expression: real-time analysis in single cells. *Cell* **116**, 683–698 (2004).
40. Chong, S. et al. Imaging dynamic and selective low-complexity domain interactions that control gene transcription. *Science* **361**, eaar2555 (2018).
41. Lyons, H. et al. Functional partitioning of transcriptional regulators by patterned charge blocks. *Cell* **186**, 327–345 e328 (2023).
42. Cardinaux, J. R. et al. Recruitment of CREB binding protein is sufficient for CREB-mediated gene activation. *Mol. Cell. Biol.* **20**, 1546–1552 (2000).
43. Chen, Z. et al. Discovery of CBPD-268 as an exceptionally potent and orally efficacious CBP/p300 PROTAC degrader capable of achieving tumor regression. *J. Med. Chem.* **67**, 5275–5304 (2024).
44. Knutson, S. K. et al. Durable tumor regression in genetically altered malignant rhabdoid tumors by inhibition of methyltransferase EZH2. *Proc. Natl Acad. Sci. USA* **110**, 7922–7927 (2013).
45. Soucy, T. A. et al. An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. *Nature* **458**, 732–736 (2009).
46. Webb, T., Craigon, C. & Ciulli, A. Targeting epigenetic modulators using PROTAC degraders: Current status and future perspective. *Bioorg. Med. Chem. Lett.* **63**, 128653 (2022).
47. Dale, B. et al. Targeting triple-negative breast cancer by a novel proteolysis targeting chimera degrader of enhancer of zeste homolog 2. *ACS Pharmacol. Transl. Sci.* **5**, 491–507 (2022).
48. Sato, R. et al. Sterol-dependent transcriptional regulation of sterol regulatory element-binding protein-2. *J. Biol. Chem.* **271**, 26461–26464 (1996).
49. Hsu, J. H. et al. EED-targeted PROTACs degrade EED, EZH2, and SUZ12 in the PRC2 complex. *Cell Chem. Biol.* **27**, 41–46 e17 (2020).
50. Chen, H. A. et al. Senescence rewires microenvironment sensing to facilitate antitumor immunity. *Cancer Discov.* **13**, 432–453 (2023).
51. Mok, E. H. K. et al. Caspase-3-induced activation of SREBP2 drives drug resistance via promotion of cholesterol biosynthesis in hepatocellular carcinoma. *Cancer Res.* **82**, 3102–3115 (2022).
52. Gao, S. et al. CRISPR screens identify cholesterol biosynthesis as a therapeutic target on stemness and drug resistance of colon cancer. *Oncogene* **40**, 6601–6613 (2021).
53. Kong, Y. et al. Inhibition of cholesterol biosynthesis overcomes enzalutamide resistance in castration-resistant prostate cancer (CRPC). *J. Biol. Chem.* **293**, 14328–14341 (2018).
54. Shimada, K. et al. Global survey of cell death mechanisms reveals metabolic regulation of ferroptosis. *Nat. Chem. Biol.* **12**, 497–503 (2016).
55. Li, W., Liang, L., Liu, S., Yi, H. & Zhou, Y. FSP1: a key regulator of ferroptosis. *Trends Mol. Med.* **29**, 753–764 (2023).
56. Sun, H. et al. p53 transcriptionally regulates SQLE to repress cholesterol synthesis and tumor growth. *EMBO Rep.* **22**, e52537 (2021).
57. Muta, Y. et al. Enhanced SREBP2-driven cholesterol biosynthesis by PKC λ /iota deficiency in intestinal epithelial cells promotes aggressive serrated tumorigenesis. *Nat. Commun.* **14**, 8075 (2023).
58. Zhang, T., Gong, Y., Meng, H., Li, C. & Xue, L. Symphony of epigenetic and metabolic regulation-interaction between the histone methyltransferase EZH2 and metabolism of tumor. *Clin. Epigenetics* **12**, 72 (2020).
59. Niccolai, R. et al. Cholesterol biosynthesis as a drug-induced vulnerability in diffuse large B cell lymphoma insensitive to EZH2 inhibition. *Neoplasia* **70**, 101243 (2025).
60. Yang, P. M., Hong, Y. H., Hsu, K. C. & Liu, T. P. p38 α /S1P/SREBP2 activation by the SAM-competitive EZH2 inhibitor GSK343 limits its anticancer activity but creates a druggable vulnerability in hepatocellular carcinoma. *Am. J. Cancer Res.* **9**, 2120–2139 (2019).
61. Kamisuki, S. et al. A small molecule that blocks fat synthesis by inhibiting the activation of SREBP. *Chem. Biol.* **16**, 882–892 (2009).
62. Zheng, L., Li, L., Lu, Y., Jiang, F. & Yang, X. A. SREBP2 contributes to cisplatin resistance in ovarian cancer cells. *Exp. Biol. Med.* **243**, 655–662 (2018).
63. Cha, T. L. et al. Akt-mediated phosphorylation of EZH2 suppresses methylation of lysine 27 in histone H3. *Science* **310**, 306–310 (2005).
64. Hao, A., Wang, Y., Stovall, D. B., Wang, Y. & Sui, G. Emerging roles of lncRNAs in the EZH2-regulated oncogenic network. *Int. J. Biol. Sci.* **17**, 3268–3280 (2021).
65. Wang, J. & Wang, G. G. No easy way out for EZH2: its pleiotropic, noncanonical effects on gene regulation and cellular function. *Int. J. Mol. Sci.* **21**, E9501 (2020).
66. Yi, Y. et al. A dual role of EZH2 in regulating A-to-I RNA editing and mRNA stability through ADAR. *Nat. Commun.* <https://doi.org/10.1038/s41467-026-71207-3> (2026).
67. Song, J. et al. Structural basis for inactivation of PRC2 by G-quadruplex RNA. *Science* **381**, 1331–1337 (2023).
68. Kim, E. et al. Phosphorylation of EZH2 activates STAT3 signaling via STAT3 methylation and promotes tumorigenicity of glioblastoma stem-like cells. *Cancer Cell* **23**, 839–852 (2013).
69. Park, K. S. et al. Targeted degradation of PRC1 components, BMI1 and RING1B, via a novel protein complex degrader strategy. *Adv. Sci.* **10**, e2205573 (2023).
70. Xiong, Y. et al. Bridged proteolysis targeting chimera (PROTAC) enables degradation of undruggable targets. *J. Am. Chem. Soc.* **144**, 22622–22632 (2022).

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Methods

All animal experiments followed Institutional Animal Care and Use Committee protocols. The Jackson Laboratory provided NOD/SCID/IL2Rgamma^{null} (NSG) and C57BL/6 mice. This study did not involve patient recruitment or clinical data use. The University of North Carolina Tissue Procurement Facility provided the de-identified human specimens (protocol UNC-LCCC-9001).

Cell lines and tissue culture

The used cell lines included Huh7 (JCRB Cell Bank, JCRB0403), HepG2 (American Tissue Culture Collection (ATCC), HB-8065), Hep3B (ATCC, HB-8064), SNU-423 (ATCC, CRL-2238), SNU-475 (ATCC, CRL-2236), HepaRG (Lonza, NSHPRG), THLE-2 (ATCC, CRL-2706), SU-DHL-6 (ATCC, CRL-2959), OCI-LY1 (DSMZ, ACC 722), WEHI-231 (ATCC, CRL-1702), A20 (ATCC, TIB-208), 22Rv1 (ATCC, CRL-2505), LNCaP (ATCC, CRL-1740), MV4;11 (ATCC, CRL-9591), HEK293 (ATCC, CRL-1573), 293T (ATCC, CRL-3216) and HeLa (ATCC, CCL-2). Cells were cultured at 37 °C with 5% CO₂. The LacO-containing U2OS 2-6-3 line was used as described previously^{39,71}. The syngeneic murine liver cancer line expressing *Nras*^{G12D} and an inducible p53 shRNA (termed 'NSP') was maintained as previously described⁵⁰.

Plasmid construction

The pCDH-EF1-MCS-IRES-neo vector (System Biosciences) for expression of HA-tagged EZH2 was previously described¹⁷. The pcDNA3.1 plasmid carrying 2×Flag-tagged SREBP2 was obtained from Addgene (cat. no. 26807). Mutagenesis was performed using the Q5 Site-Directed Mutagenesis kit (NEB, E0554). Lentiviral shRNA plasmids for KD of the human *EZH2* (TRCN0000010475 and TRCN0000040075) or *SREBP2* gene (TRCN0000020664, TRCN0000020665 and TRCN0000020667) were obtained from Sigma. For LacO-array-based imaging, the tAD of EZH2 or SREBP2 was fused in-frame to mCherry-tagged LacI in a mammalian expression vector and transiently transfected to U2OS 2-6-3 cells as described^{41,71}. For luciferase reporter assays, the HMGR promoter was cloned into the pGL3-promoter vector.

Cell proliferation assay

Cell proliferation was quantified using an automated TC10 Cell Counter (Bio-Rad) for suspension cultures or the CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega, G3582) for adherent cell cultures.

Crystal violet assay

Cells were maintained in medium supplemented with various concentrations of the indicated compounds. After a period of 2–3 weeks, colonies were visualized by staining with 0.01% (w/v) crystal violet in distilled water.

Compounds

MS7126, MS7126N1 and MS7126N2 were synthesized as described in Supplementary Note 1. MS8815, MS8815N and MS177 were synthesized as previously reported^{17,47}. Pomalidomide was obtained from AstaTech (cat. no. 36471). MLN4924 (cat. no. S7109) and A-485 (cat. no. S8740) were purchased from Selleck Chemical. Fatostatin was obtained from MedChemExpress. The enzymatic inhibitor of EZH2 (C24 (ref. 72) and EPZ-6438 (refs. 44,73)), the EED degrader (dEED; also termed PROTAC 1)⁴⁹, enzymatic inhibitor of p300/CBP (A-485)¹⁷ and p300/CBP degrader (CBPD-268)⁴³ were used as previously described.

Co-IP and western blotting

Cells were collected, lysed in buffer (50 mM Tris, pH 8.0, 120 mM NaCl, 0.5% NP-40, 0.1 mM EDTA, 10% glycerol and protease and phosphatase inhibitors) and clarified by centrifugation. For co-IP, 1 mg of protein lysate was incubated overnight at 4 °C with anti-Flag M2 magnetic beads (Sigma, M8823) or anti-HA agarose beads (Sigma, A2095) for

tagged proteins. For endogenous IP, 1 mg of total cell lysate or nuclear extract was incubated with primary antibodies overnight at 4 °C, followed by 3 h with protein A/G magnetic beads. Beads were washed and bound proteins were eluted by boiling in 2× SDS sample buffer for immunoblotting. Detailed information regarding the antibodies employed for WB is provided in Supplementary Table 9.

Cell fractionation

Cells were collected, rinsed with ice-cold 1× PBS and resuspended in 300 µl of Cytoskeleton (CSK) buffer (10 mM PIPES, pH 7.0, 300 mM sucrose, 100 mM NaCl, 3 mM MgCl₂, 0.1% Triton X-100 and protease and phosphatase inhibitors). After a 30-min incubation and centrifugation, the supernatant was collected as the soluble protein fraction, while the remaining pellet was designated as the chromatin-associated fraction. Both fractions were subsequently boiled in 2× SDS sample buffer for immunoblotting.

Quantitative PCR with reverse transcription (RT-qPCR)

Total RNA was isolated from cells or tissues utilizing the RNeasy Plus Mini kit (QIAGEN, 74134), followed by cDNA synthesis using the iScript cDNA Synthesis kit (Bio-Rad, 1708891). RT-qPCR was performed on a QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems) using SYBR Green Master Mix (Bio-Rad, 1725125). All primer sequences employed in this study are provided in Supplementary Table 9.

RNA sequencing and data analysis

RNA-seq was performed as previously described^{74,75}. Total RNA was extracted, libraries were constructed using the NEBNext Ultra II RNA Library Prep kit (NEB, E7770) and sequenced on an Illumina platform (NextSeq 500, HiSeq 4000 or NovaSeq6000). The FASTQ files were trimmed using Trim Galore and aligned to GRCh38 (GRCh38.d1.vd1.fa) using STAR v.2.7.1 with the following parameters: --outSAMunmapped Within --outSAMtype BAM Unsorted --quantMode TranscriptomeSAM. The transcript abundance was estimated with salmon (v.1.4.0) to quantify the transcriptome defined by Gencode v.22. Gene-level counts (summed across isoforms) were pre-filtered to remove low-expression genes. Only genes with a total count of no less than ten across all samples were retained for downstream analysis. DEGs were then defined using DESeq2 (v.1.38.2) in R and GSEA was conducted using the Molecular Signatures Database (MSigDB), as described previously^{74,75}.

CUT&RUN, CUT&Tag and data analysis

CUT&RUN⁷⁶ was performed according to the manufacturer's instruction (EpiCypher, 15-1116) as previously described^{17,77}. In brief, cells were washed with wash buffer (20 mM HEPES, pH 7.5, 150 mM NaCl, 0.5 mM spermidine and protease inhibitor) and incubated with activated concanavalin A-conjugated beads (Bangs Laboratories, BP531). The bead-bound cells were incubated overnight at 4 °C with the primary antibody diluted in antibody buffer (digitonin buffer with 2 mM EDTA), washed in digitonin buffer (wash buffer with digitonin) and treated with pAG-MNase to undergo targeted digestion and release solubilized chromatin. Then, DNA was purified and libraries were prepared using the NEBNext Ultra II DNA Library Prep kit (NEB, E7103).

CUT&Tag⁷⁸ was performed using EpiCypher CUTANA CUT&Tag Assay kit described previously^{71,79,80}. Cells were incubated in nuclei extraction buffer (20 mM HEPES-KOH, pH 7.9, 10 mM KCl, 0.1% Triton X-100, 0.5 mM spermidine and protease inhibitor) and then with activated concanavalin A beads (Bangs Laboratories, BP531). The bead-bound cells were incubated overnight at 4 °C with the primary antibody diluted in antibody 150 buffer (digitonin-150 buffer with 2 mM EDTA). Ice-cold secondary antibody master mix (digitonin-150 buffer with secondary antibody) was added and the samples were washed with digitonin-150 (20 mM HEPES, pH 7.5, 150 mM NaCl, 0.5 mM spermidine, protease inhibitor, 1% BSA and digitonin) and digitonin-300 buffers (20 mM HEPES, pH 7.5, 300 mM NaCl, 0.5 mM spermidine, protease

inhibitor, 1% BSA and digitonin). Following the addition of pAG-Tn5 and then tagmentation buffer (digitonin-300 buffer with 10 mM MgCl₂), the reaction was terminated by washing with TAPS buffer (10 mM TAPS, pH 8.5 and 0.2 mM EDTA). Chromatin fragments were released in SDS release buffer (10 mM TAPS, pH 8.5 and 0.1% SDS). DNA was purified and library amplification was performed using non-hot-start PCR.

The collected sequencing FASTQ files were trimmed using Trim Galore and aligned to the reference genome (hg19/hg38 or mm10) using Bowtie2 (v.2.4.4). Non-primary alignments and PCR duplicates were removed using SAMtools (v.1.10) with parameters -q 30 -F 1804 -f 2, followed by duplicate marking with Picard's MarkDuplicates function (v.2.18.2). Reads overlapping ENCODE blacklist regions were excluded using BEDTools (v.2.30.0) via the intersect function. Peak calling was performed using MACS2 (v.2.2.6) with the following parameters: macs2 callpeak -f BAMPE -g hs/mm --keep-dup 1 --cutoff-analysis -q 0.05. DeepTools (v.3.3.0) was used to generate bigWig files, heatmaps and average profiles of CUT&RUN or CUT&Tag signals. Genomic binding profiles were visualized using the 'bamCoverage' and 'computeMatrix' functions, with ENCODE blacklist regions excluded from coverage analysis. To define EZH2-'solo' and 'ensemble' peaks, unsupervised *k*-means clustering was used with the called peaks of EZH2 and H3K27me3. The R package ChIPpeakAnno (v.3.34.1)⁸¹ was used to assign peaks to nearby genes based on hg19 or mm10 gene annotation (gencode) with the following parameters: bindingType = 'fullRange', bindingRegion = c(-1, 1), ignore.peak.strand = TRUE and select = 'bestOne'. Cistrome analysis pipeline and hypergeometric optimization of motif enrichment (HOMER) were used for motif analysis.

Binding and expression target analysis

To evaluate EZH2's regulatory potential at genome-wide scales, we integrated EZH2 genomic occupancy data (CUT&RUN) with corresponding gene-expression profiles (RNA-seq) using the BETA software suite (v.2.1.1, 'plus' module)³⁵. Complete EZH2 peak sets and/or EZH2-solo peaks were utilized as input and all analyses were executed using default parameters. All genomic coordinates were mapped to the human reference genome assembly (hg19).

LacO array

U2OS 2-6-3 cells, containing a large integrated LacO array⁴¹, were seeded on coverslips and transfected with mammalian expression plasmids encoding mCherry-LacI fusion proteins using Lipofectamine 3000 Transfection Reagent (Thermo Fisher L3000015). At 48 h post-transfection, cells were fixed with 4% paraformaldehyde, permeabilized with 0.25% Triton X-100 in PBS and blocked with 4% BSA in PBS for 1 h. Samples were incubated overnight at 4 °C with primary antibody (such as rabbit anti-p300; CST, 54062). The following day, the cells were incubated with goat anti-rabbit Alexa Fluor 488 secondary antibody for 2 h and DNA was counterstained with Hoechst 33342 (Thermo Fisher, 62249). Coverslips were mounted onto glass slides using ProLong Glass Antifade Mountant (Thermo Fisher, P36982). Images were acquired using a Zeiss 880 inverted confocal microscope with Airyscan. Fluorescence signals were subsequently quantified using Fiji (ImageJ) software.

Luciferase reporter assay

Cells were co-transfected with a pGL3 luciferase reporter plasmid harbouring the HMGR promoter and mammalian expression vectors encoding 2×HA-tagged EZH2 (pCDH-EF1-IRES-neo) and/or 2×Flag-tagged SREBP2 (pCDNA3.1), using Lipofectamine 3000 Transfection Reagent. The pTK-Renilla Luc plasmid was included in all transfections as an internal normalization control. At 48 h post-transfection, luciferase activities were quantified using the Dual-Luciferase Reporter Assay System (Promega, E1910). The luminescence of the experimental firefly reporter was normalized to the *Renilla* luciferase activity to account for variations in transfection efficiency.

Cellular cholesterol quantification

Cellular cholesterol was quantified using the Total Cholesterol and Cholesteryl Ester Colorimetric/Fluorometric Assay kit (BioVision, K603-100). Cells were collected and lipids were extracted using a chloroform:isopropanol:NP-40 (7:11:0.1) mixture, followed by centrifugation. The resulting organic phase was transferred to a clean microcentrifuge tube, air-dried at 50 °C and subsequently placed under vacuum for 30 min to remove solvent. The dried lipid was resuspended in the assay buffer and vortexed until homogeneous. Total cellular cholesterol was detected and quantified via the enzymatic assay as specified in the protocol.

Protein expression and purification

A DNA fragment covering p300's cysteine-histidine-rich region 3 (p300^{CH3}; residues 1,664–1,860 of human p300 protein based on National Center for Biotechnology Information (NCBI) accession code [NM_001362843](#)) was inserted into an inhouse bacterial expression vector preceded by a His₆-MBP tag. To mimic p300 dimerization, a step necessary for p300 activation⁸², we generated a homodimeric p300^{CH3} construct by fusing a previously reported leucine zipper (LZ) sequence (HMKQLEDKVEELLSKNYHLENEVARLKKLVGER)⁸³ in-frame to the C terminus of the p300^{CH3} domain via linker sequence SMQGSGGS (denoted herein as p300^{CH3}-LZ). DNA fragments covering the tAD of human SREBP2 (SREBP2-tAD; residues 1–106 based on NCBI accession [NR_103834.2](#)) and EZH2 (EZH2-tAD; residues 135–252; NCBI accession [AY519465.1](#)) were individually inserted into a modified pRSFDuet-1 vector, preceded by a His×6-SUMO tag.

For protein expression, the expression plasmids for p300^{CH3}, p300^{CH3}-LZ, n-SREBP2-tAD and EZH2-tAD were transformed into BL21 (DE3) RIL cell strain (Agilent Technologies), respectively. The transformed cells were initially cultured in LB medium at 37 °C. After the optical density at 600 nm reached approximately 0.9, the temperature was shifted to 16 °C. The cells were then induced by 1 mM isopropyl-D-thiogalactopyranoside and continued to grow overnight. The cells were collected, lysed and disrupted in a buffer containing 50 mM Tris-HCl (pH 8.0), 1 M NaCl, 20 mM imidazole and 10% glycerol. The fusion proteins were each purified using a Ni-NTA column (Cytiva), followed by ion-exchange chromatography using a Heparin HP column (Cytiva) (for p300^{CH3} and p300^{CH3}-LZ) or a Q-HP column (Cytiva) (for SREBP2-tAD and EZH2-tAD). The individual fusion proteins were further purified through size-exclusion chromatography on a HiLoad 16/600 Superdex 200 pg column (Cytiva) pre-equilibrated with a buffer containing 25 mM Tris-HCl (pH 8.0), 5% glycerol, 250 mM NaCl and 5 mM dithiothreitol (DTT).

Analytical size-exclusion chromatography

Analytical size-exclusion chromatography analyses were performed on a Superdex 200 increase 10/300 gl column (Cytiva) at a flow rate of 0.5 ml min⁻¹ in a buffer containing 25 mM Tris-HCl (pH 8.0), 5% glycerol, 250 mM NaCl and 5 mM DTT. Each injected sample contained 500 µl of individual proteins or protein mixture at a concentration of ~0.4 mg ml⁻¹. To analyse the complex formation, the p300^{CH3} and SREBP2-tAD proteins were mixed in a molar ratio of 1:4 and the p300^{CH3}-LZ, SREBP2-tAD and EZH2-tAD proteins were mixed in a molar ratio of 1:4:4. Fractions were collected in 500-µl aliquots and analysed using SDS-PAGE.

Selectivity assays

Methyltransferase activities of PRMT1, PRMT4, G9a, GLP, SUV39H1, SUV420H1, SETDB1, SETD2, SETD7 and trimeric MLL1, pentameric MLL3, trimeric EZH2 complexes were measured by optimized radio-metric assays using specific substrate for each methyltransferase as previously reported⁸⁴. In these assays, the transfer of ³H-methyl from ³H-S-adenosylmethionine (³H-SAM) to substrates was monitored at S-adenosylmethionine (SAM) concentrations corresponding to their

previously reported K_m values. Enzyme concentrations were chosen based on previous optimizations⁸⁴. Test compounds were added at final concentrations of 10 and 50 μ M from 100% DMSO stock solutions, with a final DMSO concentration of 5% in all reactions. Signals of the control wells containing 5% DMSO without compound were defined as 100% activity. Reactions were quenched with 10 μ l of 7.5 M guanidine hydrochloride, diluted with 180 μ l of 20 mM Tris-HCl (pH 8.0) and transferred to clear 96-well plates for detection. Incorporation of the tritiated methyl group into biotinylated substrates was monitored by scintillation proximity assay with streptavidin-coated scintillant beads.

Compounds were pre-incubated with enzyme for 2 h at 23 °C before start of the reaction by addition of substrate and SAM. Reaction times varied according to the optimal conditions established for each enzyme to maintain the linearity of initial velocities. Reactions (20 μ l final volume) were carried out using either 20 mM Tris (pH 8.0) with 5 mM DTT or potassium phosphate buffer, both supplemented with 0.01% Triton X-100. To determine half-maximal inhibitory concentration (IC_{50}) value of EZH2, radiometric assays were performed as described above at the K_m of both substrates for each protein⁸⁴. Enzyme–compound mixtures were pre-incubated for 2 h at 23 °C and reactions were initiated by addition of substrate and SAM. Assays were carried out in either 20 mM Tris-HCl (pH 8.0) supplemented with 5 mM DTT or potassium phosphate buffer containing 0.01% Triton X-100, in a final reaction volume of 20 μ l. Dose–response data were analysed and fit to nonlinear regression using a four-parameter logistic model in GraphPad Prism and IC_{50} values were determined with the bottom and top constrained to 0% and 100% activity, respectively.

Mouse pharmacokinetic study

The EZH2 PROTAC MS7126 (in its HCl salt form) was prepared in 7.5% NMP, 7.5% solutol, 30% PEG-400 and 55% normal saline. Male Swiss albino mice were administered MS7126 via a single i.p. injection at a dosage of 25 mg kg⁻¹. Blood samples were collected, plasma was collected by centrifugation and plasma concentrations of MS7126 were quantified using a fit-for-purpose LC–MS/MS method. PK parameters were derived from plasma concentration–time data via nonlinear regression analysis using GraphPad Prism software.

Tumour grafts and treatment

Human liver cancer cells (3×10^6 Huh7 or 1×10^6 HepG2 cells) were subcutaneously implanted into the flanks of 6–8-week-old NSG mice, while syngeneic murine liver tumour cells (1×10^6) were subcutaneously transplanted into C57BL/6 mice. Both female and male mice were used. Investigators were blinded to the allocation of mice and outcome assessment, which was conducted by the core staff. Once tumours reached an average volume of 50 mm³, mice were randomized into treatment cohorts and administered either vehicle or the studied compound via i.p. injection twice daily. MS7126 was formulated in 7.5% NMP, 7.5% solutol HS-15, 30% PEG-400 and 55% normal saline, and MS8815 was formulated in 5% NMP, 5% solutol HS-15 and 90% normal saline; C24 was prepared in 5% NMP, 5% solutol HS-15, 30% PEG-400 and 60% captisol (20% w/v). For combination treatment, doxorubicin was administered i.p. once weekly for a total of two doses. Tumour dimensions were measured using callipers and volumes were calculated according to the formula $0.5 \times (\text{length} \times \text{width}^2)$. The end point was overall tumour volume per animal reaching 2,000 mm³.

Statistics and reproducibility

Experimental values are presented as the mean $\pm$ s.d. or mean $\pm$ s.e.m. of at least three independent experiments, unless otherwise specified. Statistical analyses were conducted using Microsoft Excel, GraphPad Prism v.10 or R statistical language. For comparisons between two independent groups with assumed normal distributions, P values were determined using an unpaired, two-tailed Student's t -test. To evaluate differences in gene expression levels between tumours and matched

healthy tissues of the TCGA patient cohort, a one-way ANOVA was employed. For non-parametric comparisons of overall expression levels across treatment groups (based on DEGs), P values were calculated using a two-sided Wilcoxon rank-sum test. Tumour growth kinetics were analysed via two-way ANOVA or mixed effects analysis, and the Kaplan–Meier survival curve of patients with HCC was assessed using a log-rank test. Significance levels are denoted as * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; and **** $P < 0.0001$. $P < 0.05$ was defined as the threshold for statistical significance and NS denotes not significant. No statistical methods were utilized to pre-determine sample sizes. No animals or data points were excluded from the analyses. Data are representative of at least two independent experiments with consistent results.

Reporting summary

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

Data availability

The genomic datasets generated in this study, comprising RNA-seq, CUT&RUN and CUT&Tag data, have been deposited at the NCBI Gene Expression Omnibus (GEO) under accession number [GSE287355](#). Publicly available gene-expression and genomic profiling datasets used in this study were retrieved from The Cancer Genome Atlas via cBioPortal, Genotype-Tissue Expression Portal and GEO under accession numbers [GSE29611](#), [GSE32465](#), [GSE205107](#), [GSE85558](#) and [GSE180448](#). All other data supporting the findings of this study are available from the corresponding author on reasonable request. Source data are provided with this paper.

Code availability

This study does not report any original code.

References

- Ahn, J. H. et al. The phenylalanine-and-glycine repeats of NUP98 oncofusions form condensates that selectively partition transcriptional coactivators. *Mol. Cell* **85**, 708–725 e709 (2025).
- Yang, X. et al. Structure-activity relationship studies for enhancer of zeste homologue 2 (EZH2) and enhancer of zeste homologue 1 (EZH1) inhibitors. *J. Med. Chem.* **59**, 7617–7633 (2016).
- Knutson, S. K. et al. A selective inhibitor of EZH2 blocks H3K27 methylation and kills mutant lymphoma cells. *Nat. Chem. Biol.* **8**, 890–896 (2012).
- Yu, X. et al. A selective WDR5 degrader inhibits acute myeloid leukemia in patient-derived mouse models. *Sci. Transl. Med.* **13**, eabj1578 (2021).
- Cai, L. et al. ZFX mediates non-canonical oncogenic functions of the androgen receptor splice variant 7 in castrate-resistant prostate cancer. *Mol. Cell* **72**, 341–354 e346 (2018).
- Meers, M. P., Bryson, T. D., Henikoff, J. G. & Henikoff, S. Improved CUT&RUN chromatin profiling tools. *Elife* **8**, e46314 (2019).
- Zhao, S. et al. TNRC18 engages H3K9me3 to mediate silencing of endogenous retrotransposons. *Nature* **623**, 633–642 (2023).
- Kaya-Okur, H. S. et al. CUT&Tag for efficient epigenomic profiling of small samples and single cells. *Nat. Commun.* **10**, 1930 (2019).
- Yu, Y. et al. Pharmacologic degradation of WDR5 suppresses oncogenic activities of SS18::SSX and provides a therapeutic of synovial sarcoma. *Sci. Adv.* **11**, eads7876 (2025).
- Li, D. et al. BAHCC1 binds H4K20me1 to facilitate the MCM complex loading and DNA replication. *Nat. Commun.* **16**, 5502 (2025).
- Zhu, L. J. et al. ChIPpeakAnno: a Bioconductor package to annotate ChIP-seq and ChIP-chip data. *BMC Bioinform.* **11**, 237 (2010).
- Ortega, E. et al. Transcription factor dimerization activates the p300 acetyltransferase. *Nature* **562**, 538–544 (2018).

83. Zeng, X., Herndon, A. M. & Hu, J. C. Buried asparagines determine the dimerization specificities of leucine zipper mutants. *Proc. Natl Acad. Sci. USA* **94**, 3673–3678 (1997).
84. Scheer, S. et al. A chemical biology toolbox to study protein methyltransferases and epigenetic signaling. *Nat. Commun.* **10**, 19 (2019).

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Author contributions

A.K., L.C. and G.G.W. conceived the idea, secured funding, designed and performed experiments and analysed and interpreted data. A.K. performed the majority of functional studies including the genomics (CUT&RUN, CUT&Tag and RNA-seq), molecular biology and in vitro and in vivo cancer biology experiments while B.P. and A.K. performed genomic data analysis under the guidance of L.C. and G.G.W. X.Y., X.G., Y.X. and H.U.K. conducted the compound synthesis and chemical biology studies under the direction of J.J. F.T. carried out the in vitro compound selectivity assays under the direction of M.V. N.K performed the protein purification and complex characterization under the direction of J.S. C.X. and Z.H. participated in the compound treatment studies. A.K., L.C. and G.G.W. prepared the manuscript with input from all authors.

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Competing interests

J.J. is a co-founder and equity shareholder in Cullgen., a scientific co-founder and scientific advisory board member of Onsero Therapeutics, and a consultant for Cullgen, EpiCypher, Accent Therapeutics and Tavotek Biotherapeutics. The Jin laboratory received research funds from Celgene Corporation, Levo Therapeutics, Cullgen and Cullinan Oncology. The other authors declare no competing interests.

Additional information

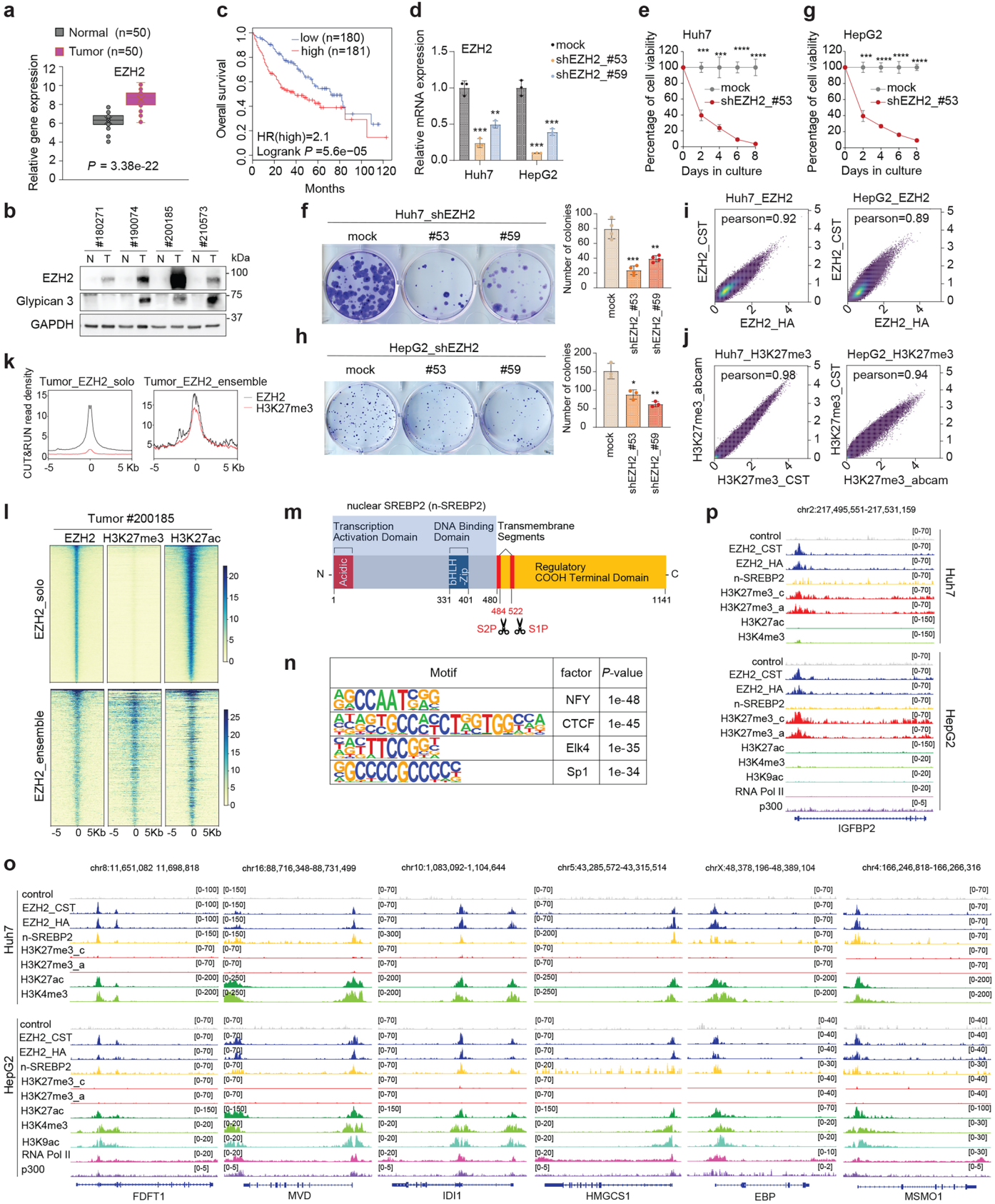
Extended data is available for this paper at <https://doi.org/10.1038/s41556-026-02048-x>.

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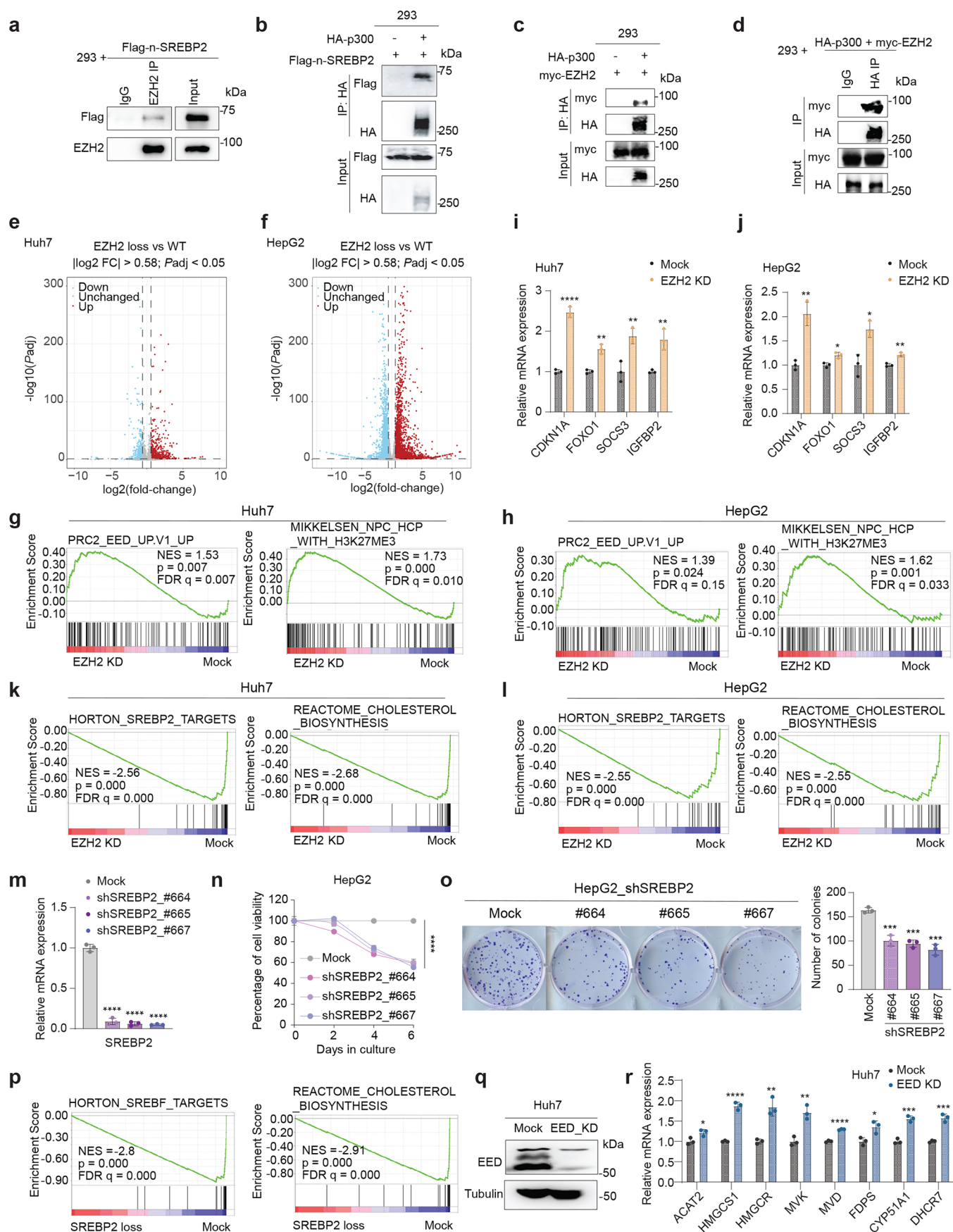
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Extended Data Fig. 1 | See next page for caption.

Extended Data Fig. 1 | EZH2 non-canonically interacts with SREBP2, exhibiting a genome-wide co-localization pattern, notably at the mevalonate (MVA) pathway genes, in liver cancer cells. (a) Box plot showing *EZH2* expression levels in primary tumours versus matched normal tissues of The Cancer Genome Atlas (TCGA) cohort of hepatocellular carcinoma (HCC) patients ($n = 50$). The y-axis shows relative gene expression normalized counts (median $\pm$ s.e.m.; box plot boundaries indicate the 25th–75th percentiles, centre lines indicate the median, and whiskers indicate $1.5 \times$ the interquartile range). P value was calculated using an unpaired, two-tailed Student's t -test. (b) Western blotting (WB) of the indicated proteins in tumour samples (T) and matched adjacent normal tissues (N) from four HCC patients. Glypican 3 serves as a marker for HCC, while GAPDH acts as a loading control. (c) Kaplan–Meier curve showing the overall survival of HCC patients based on *EZH2* expression in tumours. *EZH2* expression levels were extracted from RNA-seq data of the TCGA and Genotype-Tissue Expression (GTEx) projects. The log-rank test was used for P value calculation. (d) RT–qPCR for *EZH2* in Huh7 or HepG2 cells post-transduction with either scrambled shRNA (mock) or an *EZH2*-targeting shRNA for its knockdown (KD). Data represents the mean $\pm$ SD of relative *EZH2* expression levels, normalized to *GAPDH* and to mock-treated samples ($n = 3$ independent experiments). P value was calculated using an unpaired, two-tailed Student's t -test (**, $P < 0.01$; ***, $P < 0.001$). (e–h) Tumour cell proliferation (e,g) and representative colony-forming staining images (f,h; left) and quantification (f,h; right) using Huh7 (e,f; $n = 4$ independent experiments) and HepG2 cells (g,h; $n = 3$ independent experiments), either mock-treated or

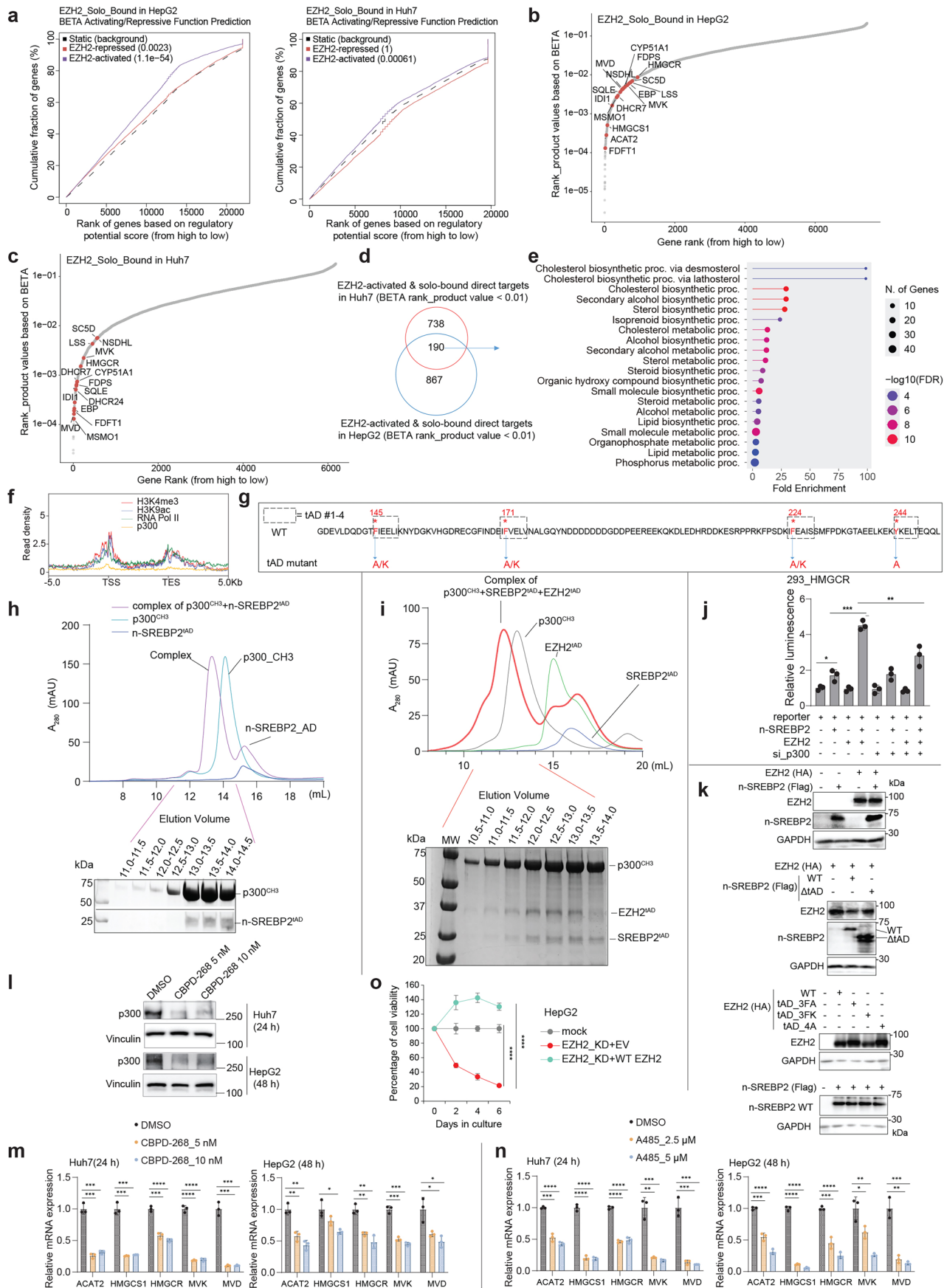
with *EZH2* KD. Cell proliferation (e,g) and colony number (f,h; right) data represent the mean $\pm$ SD, normalized to the mock-treated samples. P value was calculated using an unpaired, two-tailed Student's t -test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$). (i–j) Correlation plot using the genome-wide CUT&RUN profiles of *EZH2* (i) or H3K27me3 (j), generated with the two indicated independent antibodies in Huh7 (left) or HepG2 (right) cells. The Pearson correlation coefficient score is labelled at the top of each plot. CST (Cell Signalling Technology, Inc.) and Abcam refer to the commercial vendors. (k–l) Averaged read intensities (k) and heatmaps (l) of the indicated CUT&RUN signals at *EZH2*-solo or *EZH2*-ensemble peaks in a primary HCC patient tumour sample. (m) Schematic of full-length SREBP2 protein domain architecture, which consists of an N-terminal transcription activation domain (tAD), a basic helix-loop-helix leucine zipper (bHLH-Zip) DNA-binding domain, a central region with two hydrophobic transmembrane segments, and a C-terminal regulatory domain. Highlighted in blue is the activated nuclear SREBP2 (n-SREBP2) covering the tAD and DNA-binding domains, which is translocated to the nucleus and functions as a transcription factor (TF). (n) Enrichment of the indicated motif at *EZH2*-solo binding peaks in Huh7 and HepG2 cells as discovered by HOMER. (o–p) Integrative Genomics Viewer (IGV) tracks showing the indicated factor binding signals at either a suite of mevalonate pathway genes (o) or a classic *EZH2*:PRC2 target gene, *IGFBP2* (p) in Huh7 (top) and HepG2 (bottom) cells. Unprocessed blots, source numerical data, and P values are available in the Source data file.



Extended Data Fig. 2 | See next page for caption.

Extended Data Fig. 2 | EZH2 and SREBP2 both potentiate the expression of MVA pathway genes, promoting the liver cancer cell growth. (a) Co-IP of endogenous EZH2 and Flag-tagged n-SREBP2 transduced in HEK293 cells using an anti-EZH2 antibody. (b) Co-IP of HA-tagged p300 and Flag-tagged n-SREBP2 in HEK293 cells using an anti-HA antibody. (c–d) Co-IP of HA-tagged p300 and Myc-tagged EZH2 in HEK293 cells using an anti-HA antibody. (e–f) Volcano plot showing the expression of all transcripts in Huh7 (e) or HepG2 cells (f) with *EZH2* KD versus wild-type (WT) controls, based on RNA-seq. The threshold for differentially expressed genes (DEGs), either downregulated (blue) or upregulated (red) in cells with *EZH2* KD compared to mock controls, is set at an adjusted *P*-value (*P*_{adj}) value < 0.05 and absolute log₂-converted fold-change value ($\log_2|FC|$) > 0.58. (g–h) GSEA showing that *EZH2* KD is positively correlated with the reactivation of PRC2 target genes and H3K27me₃-associated genes in Huh7 (g) and HepG2 (h) cells, based on RNA-seq. NES, normalized enrichment score. (i–j) RT–qPCR for the indicated genes following *EZH2* KD in Huh7 (i) and HepG2 (j) cells. Data represents the mean ± SD of relative transcription levels, normalized to an internal control and mock-treated samples (*n* = 3 independent experiments). *P*-value was calculated using an unpaired, two-tailed Student's *t*-test (*, *P* < 0.05; **, *P* < 0.01; ****, *P* < 0.0001). (k–l) GSEA showing that *EZH2* KD is negatively correlated with the overall transcription of the indicated SREBP- or

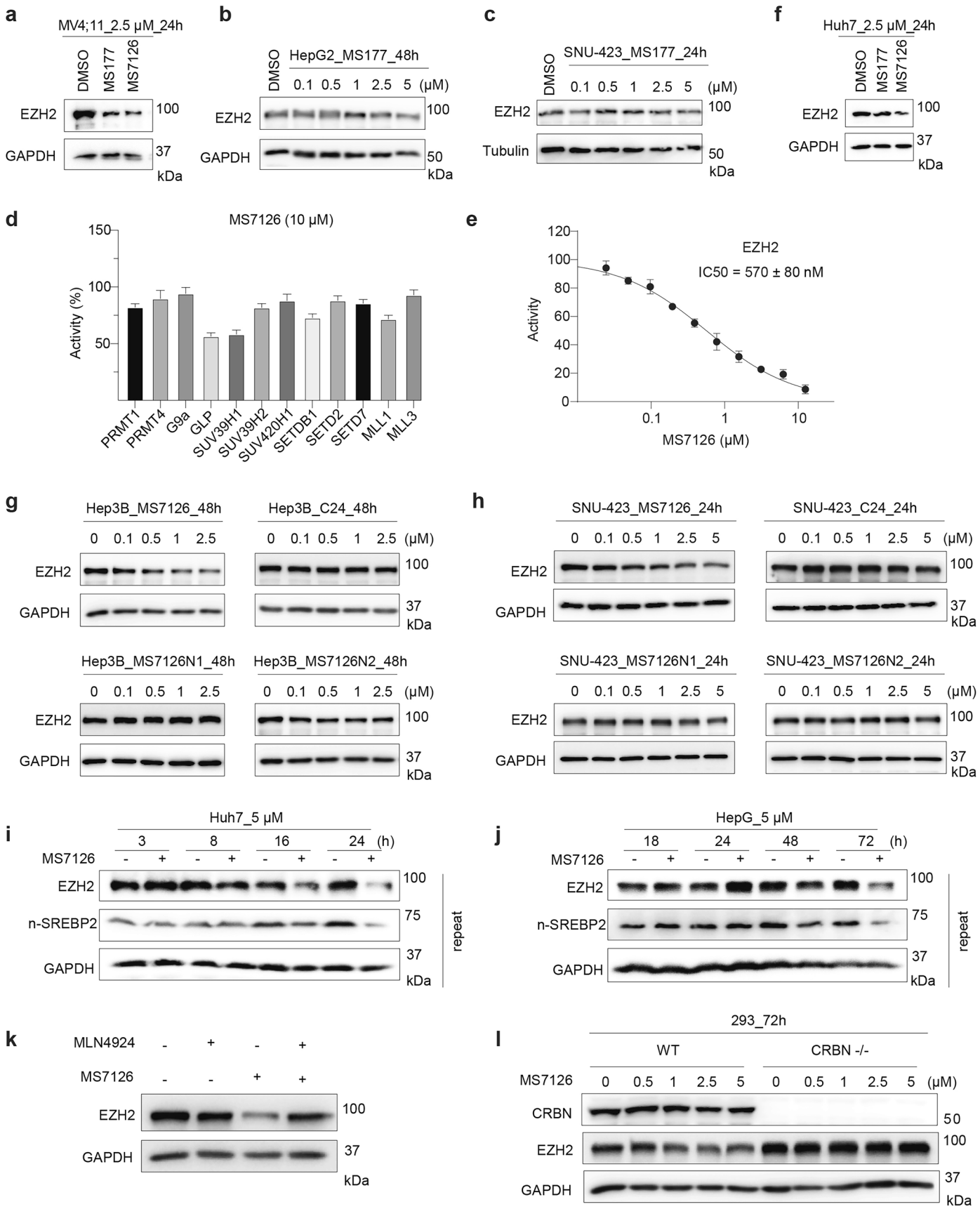
cholesterol biosynthesis-related gene sets in Huh7 (k) and HepG2 (l) cells. (m) RT–qPCR for *SREBP2* after its KD using independent shRNAs (#664, #665, and #667) in HepG2 cells. Data represents the mean ± SD of relative transcription levels, normalized to an internal control and mock-treated samples (*n* = 3 independent experiments). *P*-value was calculated using an unpaired, two-tailed Student's *t*-test (****, *P* < 0.0001). (n–o) Cell proliferation assay (n) and representative colony-forming images (o, left) and quantification (o, right) using HepG2 cells following *SREBP2* KD or mock treatment. Data represents the mean ± SD of relative cell growth normalized to mock-treated cells (*n* = 3 independent experiments). *P*-value was calculated using an unpaired, two-tailed Student's *t*-test (****, *P* < 0.0001). (p) GSEA showing that *SREBP2* KD is negatively correlated with the overall transcription of the indicated SREBP- or cholesterol biosynthesis-related gene sets in HepG2 cells. (q–r) WB of the indicated protein (q) and RT–qPCR for the indicated gene (r) in Huh7 cells after *EED* KD versus mock. Data represents the mean ± SD of relative transcription levels, normalized to an internal control and to mock-treated samples (*n* = 3 independent experiments). *P*-value was calculated using an unpaired, two-tailed Student's *t*-test (*, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001; ****, *P* < 0.0001). Unprocessed blots, source numerical data, and *P*-values are available in the Source data file.



Extended Data Fig. 3 | See next page for caption.

Extended Data Fig. 3 | The tADs of EZH2 and n-SREBP2 form direct interaction with the CH3 domain of p300, promoting the transcription of cholesterol metabolism genes. (a) Gene activating and repressive function predictions for EZH2 in HepG2 and Huh7 cells by using the Binding and Expression Target Analysis (BETA) tool. The black dashed line indicates non-differentially expressed genes, which serve as the background. In the corresponding RNA-seq data (EZH2 KD vs. control), the purple line represents downregulated genes (EZH2-activated), while the red line represents upregulated genes (EZH2-repressed). Genes are plotted cumulatively by rank based on their regulatory potential scores from highest to lowest. *P*-values, representing the statistical significance of the upregulated and downregulated group distributions compared to the background group, were calculated using the Kolmogorov-Smirnov test. (b–c) Plots showing the ranking of individual EZH2-solo-bound genes (x-axis; from highest to lowest) in HepG2 (b) and Huh7 (c) cells, based on BETA-derived rank_product score (y-axis). MVA pathway genes are highlighted in red and labelled. (d–e) BETA-based integration of genomic EZH2-binding and transcriptomic datasets, which identified the top-ranked target genes upregulated by EZH2 and directly bound by EZH2-solo in both Huh7 and HepG2 cells (with a cutoff of BETA rank_product value < 0.01), as shown in the Venn diagram (d), followed by GO analysis of the overlapped targets (e). (f) Averaged signal intensities of H3K4me3, H3K9ac, p300, and RNA Pol II at the signature genes directly activated by EZH2 (defined in Fig. 3a) in HepG2 cells. TES, transcription end site; TSS, transcription start site. (g) Amino acid sequence of the four potential tADs in EZH2 (shown in boxes with the dashed line), and the used tAD mutants with red-highlighted aromatic residues mutated to alanine (A) or lysine (K), which include EZH2^{3FA} (F145A/F171A/F224A), EZH2^{3FK} (F145K/F171K/F224K), and EZH2^{4A} (F145A/F171A/F224A/Y244A). (h) Top: Size-exclusion chromatography using recombinantly purified protein

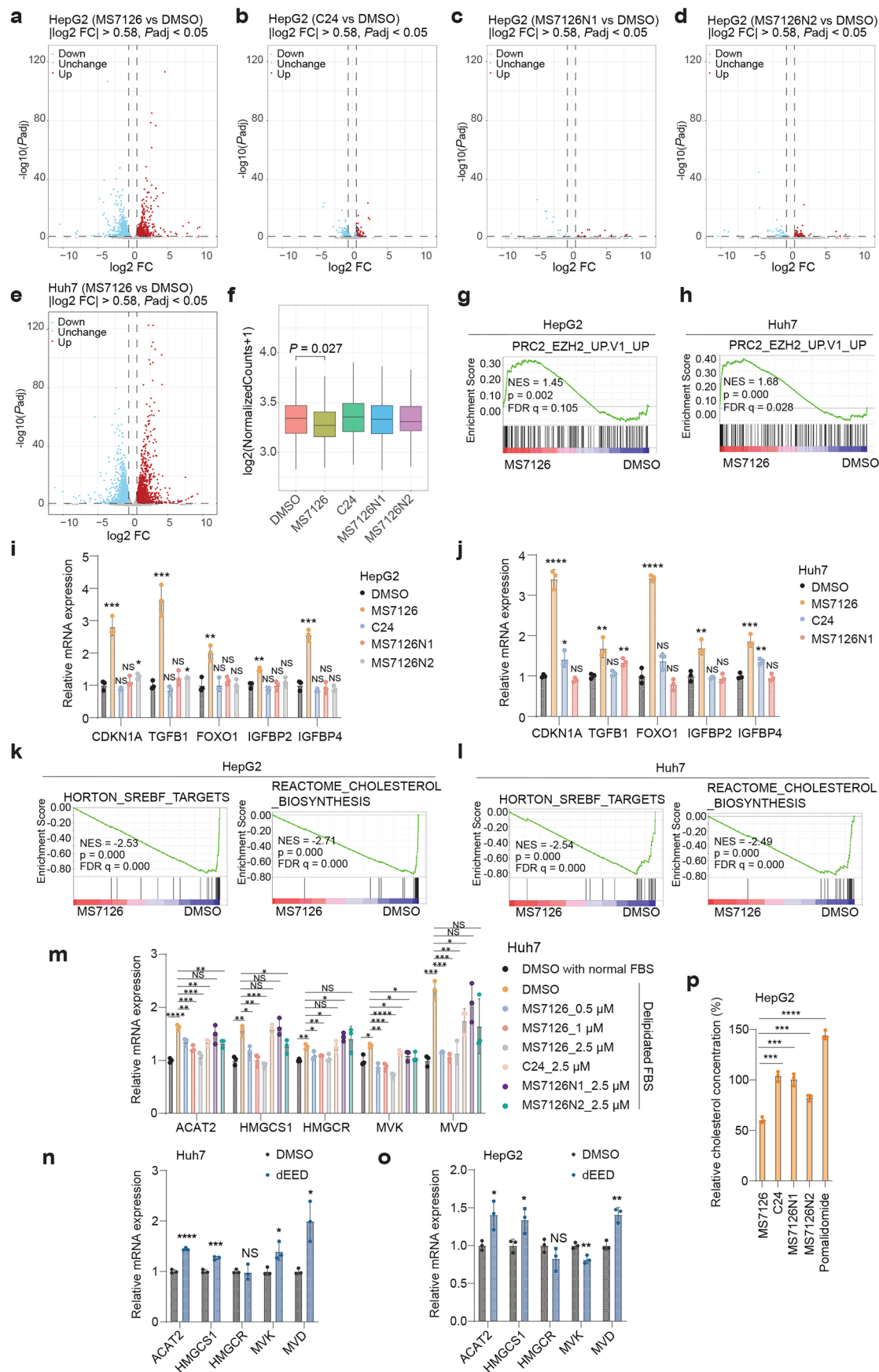
of p300^{CH3} (MBP-tagged) and n-SREBP2^{tAD} (SUMO-tagged), either individually (blue) or in a mixture (purple). Bottom: SDS-PAGE analysis of the peak fractions corresponding to the complex. (i) Top: Size-exclusion chromatography using recombinantly purified protein of the MBP-tagged p300^{CH3} dimer (also see Methods) and SUMO-tagged n-SREBP2-tAD and EZH2-tAD, either individually (shown in black, green, or blue, respectively) or in a mixture (red). Bottom: SDS-PAGE analysis of the peak fractions corresponding to the complex in the mixture. (j) Summary of *HMGCR*-luciferase reporter activation after transfection of WT n-SREBP2 or EZH2 alone, or their combination, into 293 cells, mock-treated (left) or transduced with p300-depleting RNAi (right). Relative luminescence was normalized to Renilla internal controls and to mock-transfected cells (*n* = 3 independent experiments shown as mean ± SD). *P*-value was calculated using an unpaired, two-tailed Student's *t*-test (*, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001). (k) WB for p300 in Huh7 (top) and HepG2 cells (bottom), treated with either DMSO (lane 1) or CBPD-268 (lanes 2–3) using the indicated compound concentrations and treatment durations. Vinculin serves as a loading control. (m–n) RT-qPCR for the indicated MVA pathway genes in Huh7 (left) and HepG2 cells (right), treated with CBPD-268 (m) or A-485 (n) and evaluated at the indicated compound doses and time points relative to DMSO controls. Data represents the mean ± SD of relative transcript levels normalized to an internal control and DMSO-treated samples (*n* = 3 independent experiments). *P*-value was calculated using an unpaired, two-tailed Student's *t*-test (*, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001; ****, *P* < 0.0001). (o) Proliferation assays comparing mock-treated HepG2 cells with EZH2-KD cells rescued with either an empty vector (EV) or WT EZH2 (*n* = 3 independent experiments; data are presented as mean ± SD). *P*-value was calculated using an unpaired, two-tailed Student's *t*-test (****, *P* < 0.0001). Unprocessed gels and blots, source numerical data, and *P*-values are available in the Source data file.



Extended Data Fig. 4 | See next page for caption.

Extended Data Fig. 4 | EZH2-targeting PROTACs not only deplete EZH2 but also collaterally degrade SREBP2 via a bystander mechanism in liver cancer cells. (a) WB of EZH2 and GAPDH in MV4;11 MLL-r AML cells after the indicated compound treatment for 24 h. (b–c) WB assessing EZH2 protein levels in HepG2 (b) and SNU-423 liver cancer cells (c) following the indicated MS177 treatment. (d) Selectivity of MS7126 (10 μ M) against a panel of 13 methyltransferases, measured via radioactive methyltransferase assays. Results shown are the mean values $\pm$ SD from three replicated experiments. (e) Effect of MS7126 on inhibiting EZH2 catalytic activity across multiple concentrations, measured via radioactive methyltransferase assays. IC₅₀ results shown are the mean values $\pm$ SD from triplicate experiments. (f) WB of EZH2 in Huh7 liver cancer cells after

the indicated compound treatment. (g–h) WB assessing EZH2 protein levels in Hep3B (g) and SNU-423 liver cancer cells (h) following the indicated compound treatment. (i–j) WB of EZH2, n-SREBP2, and GAPDH in Huh7 (i) and HepG2 cells (j) treated with 5 μ M MS7126 for the indicated time points. These panels and main Fig. 4d, e are representative results from independent experiments. (k) WB of EZH2 in Huh7 cells pre-treated with DMSO or MLN4924 (0.4 μ M) for 2 h, followed by treatment with DMSO or MS7126 (2.5 μ M) for 24 h. (l) WB of CRBN, EZH2, and GAPDH in paired WT (left) and CRBN-null (right; CRBN^{-/-}) 293 cells following treatment with increasing concentrations of MS7126 for 72 h. Unprocessed blots, source numerical data, and *P*-values are available in the Source data file.



Extended Data Fig. 5 | See next page for caption.

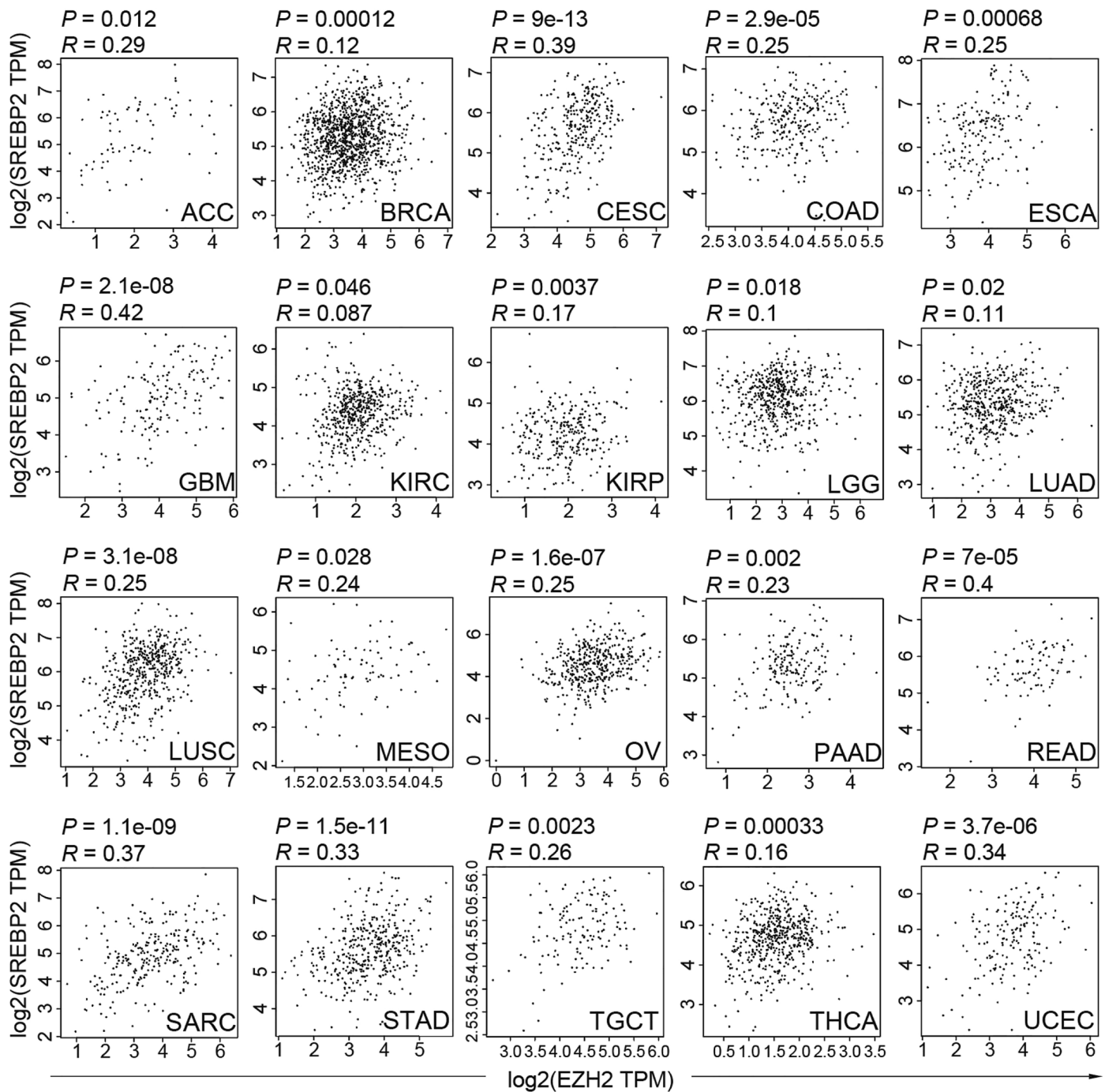
Extended Data Fig. 5 | Compared with matched non-PROTAC analogues and an -targeting PROTAC, the EZH2 PROTACs uniquely and superiorly suppress MVA pathway genes and cholesterol biosynthesis in liver cancer cells. (a–d) Volcano plots showing transcript expression based on RNA-seq of HepG2 cells following a 48-hour treatment with 5 μ M MS7126 (**a**), C24 (**b**), MS7126N1 (**c**), or MS7126N2 (**d**) in comparison to DMSO. The threshold for DEGs (blue: downregulated; red: upregulated) was set at an adjusted P -adj < 0.05 and $|\text{Log2FC}| > 0.58$. **(e)** Volcano plot showing transcript expression based on RNA-seq of Huh7 liver cancer cells following a 24-hour treatment with 5 μ M MS7126 in comparison to DMSO. Thresholds for DEGs are the same as in **(a–d)**. **(f)** Box plot showing overall expression levels across the indicated cell treatment groups using DEGs downregulated in both Huh7 and HepG2 cells following *EZH2* KD, based on RNA-seq ($n = 2$ replicated experiments). The boundaries of the box plot indicate the 25th and 75th percentiles, the centre lines indicate the median, and the whiskers indicate $1.5 \times$ the interquartile range. A two-sided Wilcoxon rank-sum test was used for P -value calculation. **(g–h)** GSEA showing that MS7126 versus DMSO treatment is positively correlated with the reactivation of PRC2 target genes in HepG2 (**g**) and Huh7 (**h**) cells, based on RNA-seq. NES, normalized enrichment score. **(i–j)** RT–qPCR for known PRC2 target genes in HepG2 (**i**) and Huh7 (**j**) cells treated with 5 μ M of the indicated compound for 48 and 24 h, respectively. Data represents relative transcription levels (mean $\pm$ SD) normalized to internal

controls and DMSO-treated samples ($n = 3$ independent experiments). **(k–l)** GSEA showing that MS7126 treatment is negatively correlated with the overall transcription of SREBP targets (left) or cholesterol biosynthesis-related genes (right) in HepG2 (**k**) and Huh7 (**l**) cells. **(m)** RT–qPCR for the indicated MVA pathway genes (also see Fig. 2j) in Huh7 cells grown in medium supplemented with either normal FBS (black) or delipidated FBS, following a 24-hour treatment with MS7126 (0.5, 1, or 2.5 μ M), C24 (2.5 μ M), MS7126N1 (2.5 μ M), or MS7126N2 (2.5 μ M). Data represents relative transcription levels (mean $\pm$ SD) normalized to internal controls and DMSO-treated samples ($n = 3$ independent experiments). **(n–o)** RT–qPCR for the indicated MVA pathway genes in Huh7 (**n**) and HepG2 (**o**) cells after a 24-hour treatment with 5 μ M of EED degrader (EED PROTAC #1) in comparison to mock controls. Data represents relative transcription levels (mean $\pm$ SD) normalized to internal controls and DMSO-treated samples ($n = 3$ independent experiments). **(p)** Measurement of cholesterol concentration in HepG2 cells after 4 days of treatment with DMSO, MS7126, C24, MS7126N1, MS7126N2, or pomalidomide (5 μ M). Data represents relative cholesterol concentration (mean $\pm$ SD) normalized to DMSO-treated samples ($n = 3$ independent experiments). Statistical significance across **i–j** and **m–p** was calculated using an unpaired, two-tailed Student's t -test; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. Source numerical data and P -values are available in the Source data file.



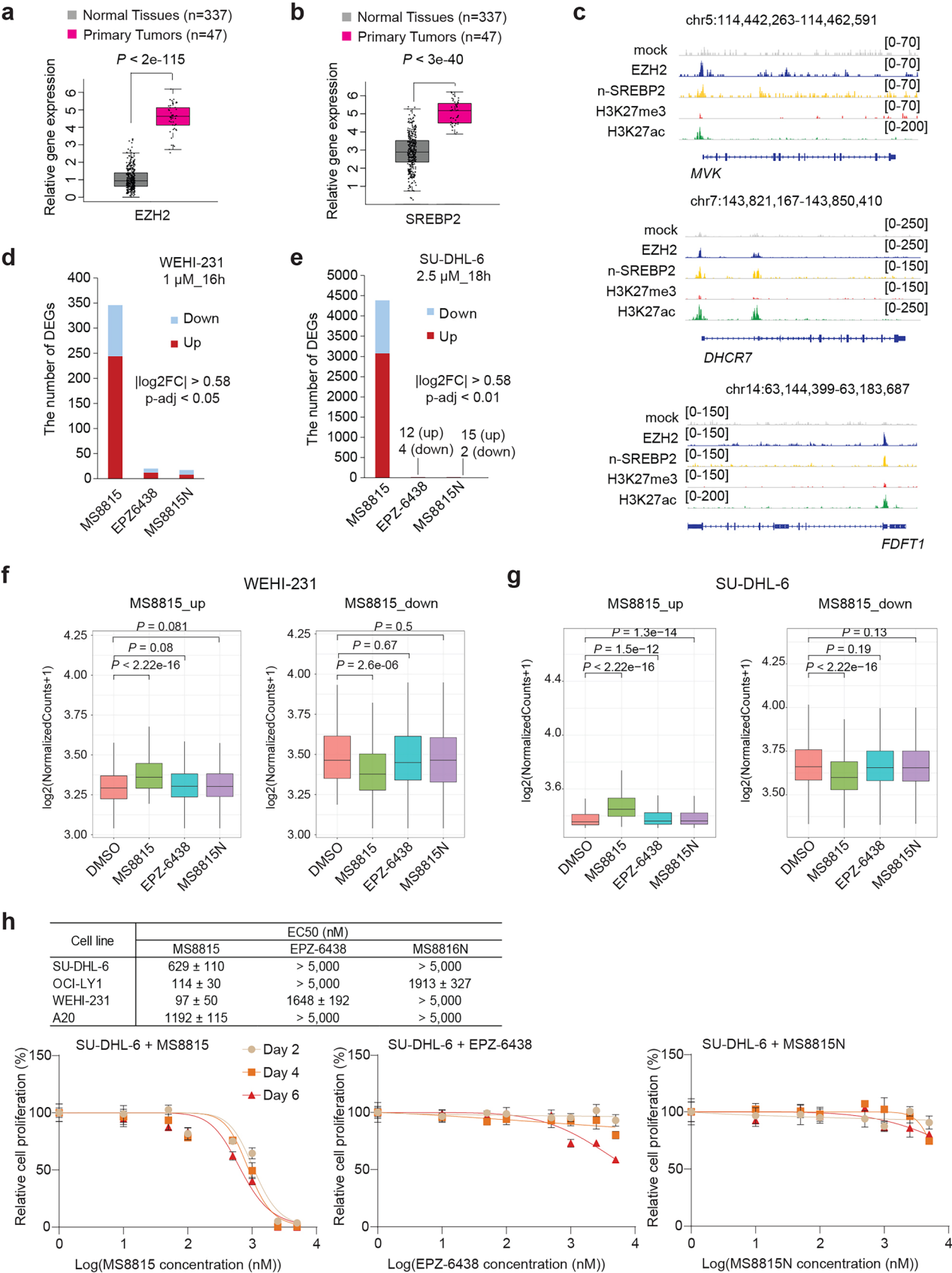
Extended Data Fig. 6 | The EZH2-targeting PROTAC exhibits superior tumour-suppressing effects in vitro and in vivo compared to EZH2 enzymatic inhibitor. (a-e) MTS assays using Huh7 (**a**), HepG2 (**b**), Hep3B (**c**), SNU-423 (**d**), and THLE-2 cells (**e**) treated with the indicated concentrations of MS7126, C24, MS7126N1, or MS7126N2 for 2, 4 or 6 days, compared to DMSO. Data represents relative cell growth (mean $\pm$ SD) normalized to DMSO-treated controls (n = 3 independent experiments). **(f-g)** Representative images of colony formation by the indicated liver cancer lines, treated with DMSO, MS7126, C24, MS7126N1, or MS7126N2 in **f**, or DMSO, MS8815, EPZ-6438, or MS8815N in **g**. **(h-i)** Representative images of colony formation (**h**) by a murine liver tumour

model carrying *Nras_G12D/shP53* (NSP), treated with the indicated compounds, and quantification of colonies (**i**). Data in (**i**) represents quantification of colony area (mean $\pm$ SD) relative to mock controls (n = 3 independent experiments). An unpaired, two-tailed Student's t-test was used for *P*-value calculation. **, *P* < 0.01; ****, *P* < 0.0001. **(j-k)** Body weight of NSG mice bearing HepG2-derived (**j**; n = 5 per group) or Huh7-derived CDX tumours (**k**; n = 3 per group), measured from the start of in vivo treatment with either vehicle or MS7126 (25 mg kg⁻¹, BID, 5 days per week). Data are presented as mean $\pm$ s.e.m. Source numerical data and *P*-values are available in the Source data file.



Extended Data Fig. 7 | Mining of TCGA patient datasets shows a positive correlation between EZH2 and SREBP2 in diverse cancers. Pearson correlation coefficient (R) between *EZH2* and *SREBP2* transcript levels in primary tumours of the indicated patient TCGA cohorts, which include Adrenocortical carcinoma (ACC), Breast invasive carcinoma (BRCA), Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), Colon adenocarcinoma (COAD), Oesophageal carcinoma (ESCA), Glioblastoma multiforme (GBM), Kidney renal

clear cell carcinoma (KIRC), Kidney renal papillary cell carcinoma (KIRP), Brain Lower Grade Glioma (LGG), Lung adenocarcinoma (LUAD), Lung squamous cell carcinoma (LUSC), Mesothelioma (MESO), Ovarian serous cystadenocarcinoma (OV), Pancreatic adenocarcinoma (PAAD), Rectum adenocarcinoma (READ), Sarcoma (SARC), Stomach adenocarcinoma (STAD), Testicular Germ Cell Tumours (TGCT), Thyroid carcinoma (THCA), and Uterine Corpus Endometrial Carcinoma (UCEC).



Extended Data Fig. 8 | See next page for caption.

Extended Data Fig. 8 | EZH2-dependent potentiation of MVA pathway genes and cholesterol biosynthesis-related programmes is applicable to B-cell lymphoma. (a–b) Box plot showing *EZH2* (a) and *SREBP2* (b) expression levels in primary tumours (right, $n = 47$) versus normal tissues (left, $n = 337$) of the TCGA diffuse large B-cell lymphoma (DLBCL) patient cohort. The y-axis shows relative gene expression normalized counts presented as median $\pm$ s.e.m. The boundaries of box plots indicate the 25th and 75th percentiles, the centre lines indicate the median, and the whiskers indicate $1.5\times$ the interquartile range. A One-way ANOVA was used for P -value calculation. **(c)** IGV tracks showing CUT&Tag signals of the indicated proteins at *MVK*, *DHCR7*, and *FDFI1* in WEHI-231 lymphoma cells. **(d–e)** Summary plots showing the total number of DEGs in WEHI-231 cells (d; following 16-hour treatment with $1\ \mu\text{M}$ MS8815, EPZ-6438, or MS8815N) and SU-DHL-6 DLBCL cells (e; following 18-hour treatment with $2.5\ \mu\text{M}$ MS8815, EPZ-6438, or MS8815N) compared to vehicle controls, as identified by RNA-seq.

(f–g) Box plots displaying the overall expression of transcripts that were either upregulated (left) or downregulated (right) following MS8815 versus DMSO treatment across the indicated sample groups of WEHI-231 cells (f) and SU-DHL-6 cells (g), based on RNA-seq ($n = 2$ replicated experiments). The boundaries of box plots indicate the 25th and 75th percentiles, the centre lines indicate the median, and the whiskers indicate $1.5\times$ the interquartile range. A two-sided Wilcoxon rank-sum test was used for P -value calculation. **(h)** Top panel: EC_{50} values of MS8815, EPZ-6438, and MS8815N in the indicated B-cell lymphoma cell line after a six-day treatment. Bottom panel: MTS assays using SU-DHL-6 cells treated with the indicated concentrations of compound (x-axis) for 2, 4, or 6 days. Data represents the mean $\pm$ SD of relative cell proliferation normalized to DMSO-treated controls ($n = 3$ independent experiments). Source numerical data and P -values are available in the Source data file.



Extended Data Fig. 9 | The EZH2–n-SREBP2–MVA pathway exists in AR-positive prostate cancer and MLL-r leukaemia. (a–b) Heatmaps (a) and averaged signal intensities (b) showing CUT&RUN or CUT&Tag signals of the indicated proteins, ± 5 kb from the centres of EZH2-solo or EZH2-ensemble peaks in MV4;11 cells. (c) Venn diagram showing the overlap between EZH2-solo peaks and n-SREBP2 peaks in MV4;11 cells. (d) IGV tracks showing the genomic signal enrichment of the indicated proteins at mevalonate pathway genes (*HMGCR*, *HMGCS1*, *ACAT2*, and *MVK*) in MV4;11 cells. (e) IGV tracks showing the genomic signal enrichment of the indicated proteins at PRC2 target genes, *BACE1* in MV4;11 cells (left) and *SLC13A5* in 22Rv1 cells (right). (f–g) RT–qPCR for MVA pathway genes in MV4;11 (f) and 22Rv1 cells (g) treated for 24 h with the indicated concentration (2.5 μ M) of MS177 or C24, relative to DMSO controls. Data represents the mean $\pm$ SD of relative transcription levels normalized to an internal control and DMSO-treated

samples ($n = 3$ independent experiments). (h) WB for EZH2 and n-SREBP2 after the indicated compound treatment in 22Rv1 and MV4;11 cells. (i–j) RT–qPCR for MVA pathway genes following treatment of Huh7 cells (i; 24 h) and HepG2 cells (j; 48 h) with the indicated concentration of fatostatin, relative to DMSO controls. Data represents the mean $\pm$ SD of relative transcription levels normalized to an internal control and DMSO-treated samples ($n = 3$ independent experiments). (k) Proliferation of Huh7 and HepG2 cells following a three-day treatment with DMSO, fatostatin alone, MS7126 alone, or their combination. Data represents the mean $\pm$ SD of relative growth normalized to DMSO-treated cells ($n = 3$ independent experiments). Statistical significance across f–g and i–k was calculated using an unpaired, two-tailed Student's *t*-test; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. Unprocessed blots, source numerical data, and *P*-values are available in the Source data file.

Reporting Summary

Nature Portfolio 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 Portfolio policies, see our [Editorial Policies](#) 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 (<i>n</i>) 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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	None of the custom softwares were used in this study. Deep sequencing data was collected by using an Illumina NextSeq500, NextSeq 1000, HiSeq 4000, or NovaSeq6000 system. The quantitative PCR was performed with an ABI 7900HT fast real-time qPCR system. For western blotting, the membranes were scanned using an ImageQuant LAS 4000 Luminescent Image Analyzer (GE Healthcare) or a ChemiDoc Imaging Systems (Bio-Rad).
Data analysis	The collected data sets were analyzed by using the following softwares and analytic tools: GraphPad Prism 10 (Version 10.2.1) ImageJ (Fiji, Version 2.14.0/1.54f) Cistrome analysis pipeline HOMER (Version 4.10.0) BEDTools (Version 2.30.0) STAR aligner (Version 2.7.1) DESeq2 (Version 1.38.2) SAMtools (Version 1.10) Bowtie2 (Version 2.4.4) MACS2 (Version 2.2.6) IGV Browser (Version 2.6.1) DeepTools (Version 3.3.0) PANTHER Picard (Version 2.20.4) Salmon (Version 1.4.0)

GEPIA2
GSEA (Version 4.1.0)
BETA (Version 2.1.1)

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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Next-generation sequencing datasets including those of RNA-seq, CUT&RUN, and CUT&Tag datasets related to this work have been deposited in the NCBI GEO under accession number GSE287355.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

n/a

Reporting on race, ethnicity, or other socially relevant groupings

n/a

Population characteristics

n/a

Recruitment

n/a

Ethics oversight

n/a

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

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](https://www.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

No statistical methods were used to predetermine the sample size. Sample size was determined based on the previously published studies and equivalent to what is routinely used for respective assays. Sample sizes are indicated for all experiments. At least 2 or 3 independent experiments were carried out for most of the assays.

Data exclusions

No data were excluded from analysis.

Replication

Experiments were reproduced in independent settings. All attempts of replication were successful and noted in the relevant figure legends.

Randomization

All the samples used in the study were randomly allocated. For the mice with tumor xenografts, they were randomized to different groups for treatment of the studied agent versus mock.

Blinding

Investigators were blinded to the group allocation of animals during the data collection. We also conducted the blinded data analyses, except when the data were not easily subjected to investigator bias.

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

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☐	☒ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Anti-alpha Tubulin antibody (DM1A), mouse monoclonal, Abcam ab7291, 1:5000 for WB
 Anti-CRBN antibody (D8H3S), rabbit monoclonal, Cell Signaling Technology 71810, 1:1000 for WB
 Anti-EZH2 antibody (clone 11), mouse monoclonal, BD 612666, 1:1000 for WB
 Anti-EZH2 antibody (D2C9), rabbit monoclonal, Cell Signaling Technology 5246, 1:100 for CUT&RUN and CUT&Tag
 Anti-FLAG M2 antibody, mouse monoclonal, Sigma-Aldrich F1804, 1:100 for Co-IP
 Anti-FLAG-HRP antibody, mouse monoclonal, Sigma-Aldrich A8592, 1:5000 for WB
 Anti-GAPDH antibody (14C10), rabbit monoclonal, Cell Signaling Technology 2118, 1:5000 for WB
 Anti-Glypican 3 antibody (SP86), rabbit monoclonal, Abcam ab95363, 1:1000 for WB
 Anti-HA-Tag antibody (C29F4), rabbit monoclonal, Cell Signaling Technology 3724, 1:5000 for WB, 1:100 for CUT&RUN
 Anti-H3 antibody, rabbit polyclonal, Cell Signaling Technology 9715, 1:5000 for WB
 Anti-H3K27ac antibody, rabbit polyclonal, Abcam ab4729, 1:100 for CUT&RUN and CUT&Tag
 Anti-H3K27me3 antibody, mouse monoclonal, Abcam ab6002, 1:100 for CUT&RUN
 Anti-H3K27me3 antibody (C36B11), rabbit monoclonal, Cell Signaling Technology 9733, 1:2000 for WB, 1:100 for CUT&RUN and CUT&Tag
 Anti-H3K27me3 antibody, rabbit polyclonal, Millipore 07-449, 1:100 for CUT&Tag
 Anti-H3K4me3 antibody, rabbit polyclonal, Abcam ab8580, 1:100 for CUT&RUN
 Mouse (G3A1) mAb IgG1 Isotype Control, monoclonal, Cell Signaling Technology 5415
 Normal Rabbit IgG, polyclonal, Cell Signaling Technology 2729
 Anti-p300 antibody (D2X6N), rabbit monoclonal, Cell Signaling Technology 54062, 1:1000 for WB, 1:1000-2000 for IF
 Anti-SREBP2 antibody, rabbit polyclonal, Abcam ab30682, 1:100 for CUT&RUN and CUT&Tag
 Anti-SREBP2 antibody, rabbit polyclonal, Cayman 10007663, 1:1000 for WB, 1:100 for CUT&RUN
 Anti-SREBP2 antibody (Clone IgG-1C6), mouse monoclonal, BD 557037, 1:1000 for WB
 Anti-EED antibody, sheep polyclonal, R&D AF5827, 1:1000 for WB
 Anti-SUZ12 antibody, rabbit polyclonal, Abcam ab12073, 1:1000 for WB
 Anti-Myc-Tag antibody (9B11), mouse monoclonal, Cell Signaling Technology 2276, 1:1000 for WB
 Anti-Vinculin antibody (E1E9V), rabbit monoclonal, Cell Signaling Technology 13901, 1:2000 for WB
 Anti-mouse IgG, HRP-linked antibody Cell Signaling Technology 7076, 1:5000 for WB
 Anti-rabbit IgG, HRP-linked antibody Cell Signaling Technology 7074, 1:5000 for WB
 Sheep IgG, HRP-conjugated antibody R&D HAF016, 1:2500 for WB
 Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488, Invitrogen A-11008, 1:1000 for IF
 Hoechst 33342, Invitrogen H3570, 1:5000 for IF

Validation

All commercial antibodies were validated as noted on the manufacturer's website.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

Huh7 JCRB Cell Bank JCRB0403
 HepG2 ATCC HB-8065
 Hep3B ATCC HB-8064
 HepaRG Lonza NSHPRG
 SNU-423 ATCC CRL-2238
 SNU-475 ATCC CRL-2236
 THLE-2 ATCC CRL-2706
 SU-DHL-6 ATCC CRL-2959
 OCI-LY1 DSMZ ACC 722
 WEHI-231 ATCC CRL-1702
 A20 ATCC TIB-208
 22Rv1 ATCC CRL-2505
 LNCaP ATCC CRL-1740
 MV4;11 ATCC CRL-9591

K562 ATCC CRL-243
 HEK293 ATCC CRL-1573
 293T ATCC CRL-3216
 HeLa ATCC CCL-2
 U2OS 2-6-3 from Janicki SM et al. Cell (2004) 116, 683–698 [PMID: 15006351].
 NrasG12D/sh_p53 (NSP) a kind gift of J Morris lab at UNC

Authentication

Authentication of cell line identities was ensured by the Tissue Culture Facility affiliated to our institute with the recommended genetic signature profiling or fingerprinting analysis.

Mycoplasma contamination

Cells were negative for mycoplasma. Every month, a routine examination of cell lines in culture for any possible mycoplasma contamination was performed using commercially available detection kits (Lonza).

Commonly misidentified lines
(See [ICLAC](#) register)

We confirm that all cell lines used in this study were tested and authenticated, and none are included in the ICLAC Register of Misidentified Cell Lines.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

NOD/SCID/IL2Rgammanull (NSG) and C57BL/6 mice (an age of 6-8 weeks) were purchased from Jackson Lab and used in this study.

Wild animals

No wild animals were used in the study.

Reporting on sex

Both female and male mice were used.

Field-collected samples

No field-collected samples were used in the study.

Ethics oversight

All animal experiments were performed in accordance with the procedures approved by the Institutional Animal Care and Use Committee at the University. Mice were housed in a germ-free environment with a 12 h light/ 12 h dark cycle, maintained at room temperature of 22±2 °C and relative humidity at 30–70%.

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

Plants

Seed stocks

n/a

Novel plant genotypes

n/a

Authentication

n/a

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.

Next-generation sequencing datasets including those of CUT&RUN and CUT&Tag datasets related to this work have been deposited in the NCBI GEO under accession number GSE287355.

Files in database submission

HepG2_control_R1.fastq.gz
 HepG2_EZH2_CST-R1.fastq.gz
 HepG2_EZH2_HA-R1.fastq.gz
 HepG2_H3K27me3_CST-R1.fastq.gz
 HepG2_H3K27me3_abcam-R1.fastq.gz
 HepG2_H3K27ac-R1.fastq.gz
 HepG2_H3K4me3-R1.fastq.gz

HepG2_SREBP2-R1.fastq.gz
 Huh7_control_R1.fastq.gz
 Huh7_EZH2_CST-R1.fastq.gz
 Huh7_EZH2_HA-R1.fastq.gz
 Huh7_H3K27me3_CST-R1.fastq.gz
 Huh7_H3K27me3_abcam-R1.fastq.gz
 Huh7_H3K27ac-R1.fastq.gz
 Huh7_H3K4me3-R1.fastq.gz
 Huh7_SREBP2-R1.fastq.gz
 Tumor_EZH2-R1.fastq.gz
 Tumor_H3K27me3-R1.fastq.gz
 Tumor_H3K27ac-R1.fastq.gz
 WEHI-231_control_R1.fastq.gz
 WEHI-231_Ezh2_R1.fastq.gz
 WEHI-231_H3K27me3_R1.fastq.gz
 WEHI-231_H3K27ac_R1.fastq.gz
 WEHI-231_Srebp2_R1.fastq.gz
 HepG2_control_R2.fastq.gz
 HepG2_EZH2_CST-R2.fastq.gz
 HepG2_EZH2_HA-R2.fastq.gz
 HepG2_H3K27me3_CST-R2.fastq.gz
 HepG2_H3K27me3_abcam-R2.fastq.gz
 HepG2_H3K27ac-R2.fastq.gz
 HepG2_H3K4me3-R2.fastq.gz
 HepG2_SREBP2-R2.fastq.gz
 Huh7_control_R2.fastq.gz
 Huh7_EZH2_CST-R2.fastq.gz
 Huh7_EZH2_HA-R2.fastq.gz
 Huh7_H3K27me3_CST-R2.fastq.gz
 Huh7_H3K27me3_abcam-R2.fastq.gz
 Huh7_H3K27ac-R2.fastq.gz
 Huh7_H3K4me3-R2.fastq.gz
 Huh7_SREBP2-R2.fastq.gz
 Tumor_EZH2-R2.fastq.gz
 Tumor_H3K27me3-R2.fastq.gz
 Tumor_H3K27ac-R2.fastq.gz
 WEHI-231_control_R2.fastq.gz
 WEHI-231_Ezh2_R2.fastq.gz
 WEHI-231_H3K27me3_R2.fastq.gz
 WEHI-231_H3K27ac_R2.fastq.gz
 WEHI-231_Srebp2_R2.fastq.gz
 22Rv1_EZH2_R1.fastq.gz
 22Rv1_SREBP2_abcam_R1.fastq.gz
 22Rv1_SREBP2_cayman_R1.fastq.gz
 22Rv1_control_R1.fastq.gz
 MV4;11_EZH2_R1.fastq.gz
 MV4;11_SREBP2_abcam_R1.fastq.gz
 MV4;11_SREBP2_cayman_R1.fastq.gz
 MV4;11_control_R1.fastq.gz
 MV4;11_H3K27me3_R1.fastq.gz
 MV4;11_H3K27ac_R1.fastq.gz
 MV4;11_control_CT_R1.fastq.gz
 22Rv1_EZH2_R2.fastq.gz
 22Rv1_SREBP2_abcam_R2.fastq.gz
 22Rv1_SREBP2_cayman_R2.fastq.gz
 22Rv1_control_R2.fastq.gz
 MV4;11_EZH2_R2.fastq.gz
 MV4;11_SREBP2_abcam_R2.fastq.gz
 MV4;11_SREBP2_cayman_R2.fastq.gz
 MV4;11_control_R2.fastq.gz
 MV4;11_H3K27me3_R2.fastq.gz
 MV4;11_H3K27ac_R2.fastq.gz
 MV4;11_control_CT_R2.fastq.gz

Genome browser session
(e.g. [UCSC](#))

not used

Methodology

Replicates

All experiments were performed with technical replicates for each condition. We used biological replicates across different experiments, such as independent liver cancer models (e.g. Huh7, HepG2 and HepB3), independent antibodies (such as endogenous EZH2 antibody and HA antibody for HA-tagged EZH2, two different SREBP2 antibodies, and two different H3K27me3 antibodies), etc.

Replicate agreement of genomic data sets was high across independent libraries.

Sequencing depth

Sequencing samples paired-end (PE) unique reads_hg19/hg38/mm10 read length
 HepG2_control 8,186,164 150
 HepG2_EZH2_CST 9,883,227 150
 HepG2_EZH2_HA 7,959,030 150
 HepG2_H3K27me3_CST 12,195,606 150
 HepG2_H3K27me3_abcam 10,326,016 150
 HepG2_H3K27ac 9,944,531 150
 HepG2_H3K4me3 11,148,424 150
 HepG2_SREBP2 11,374,076 150
 Huh7_control 11,292,867 150
 Huh7_EZH2_CST 8,603,621 150
 Huh7_EZH2_HA 7,780,545 150
 Huh7_H3K27me3_CST 7,483,196 150
 Huh7_H3K27me3_abcam 12,418,996 150
 Huh7_H3K27ac 12,278,257 150
 Huh7_H3K4me3 7,509,990 150
 Huh7_SREBP2 10,240,626 150
 Tumor_EZH2 13,025,155 150
 Tumor_H3K27me3 15,810,006 150
 Tumor_H3K27ac 15,012,985 150
 WEHI-231_control 4,679,137 150
 WEHI-231_EZH2 7,153,978 150
 WEHI-231_H3K27me3 7,112,983 150
 WEHI-231_H3K27ac 4,322,716 150
 WEHI-231_SREBP2 7,343,236 150
 22Rv1_EZH2 16,540,255 150
 22Rv1_SREBP2_abcam 20,646,063 150
 22Rv1_SREBP2_cayman 15,059,907 150
 22Rv1_control 11,582,052 150
 MV4;11_EZH2 17,007,738 150
 MV4;11_SREBP2_abcam 18,495,263 150
 MV4;11_SREBP2_cayman 17,809,734 150
 MV4;11_control 15,607,747 150
 MV4;11_H3K27me3 13,066,026 150
 MV4;11_H3K27ac 14,075,543 150
 MV4;11_control_Cut&Tag 7,519,332 150

Antibodies

EZH2 (CST5246)
 HA (CST3724)
 H3K27me3 (CST9733)
 H3K27me3 (ab6002)
 H3K27me3 (Millipore 07-449)
 H3K27ac (ab4729)
 H3K4me3 (ab8580)
 SREBP2 (ab30682)
 SREBP2 (cayman 10007663)

Peak calling parameters

Peak calling was performed using MACS2 (v2.2.6) with the following parameters: macs2 callpeak -f BAMPE -g hs/mm --keep-dup 1 --cutoff-analysis -q 0.05.

Data quality

FastQC

Software

STAR (v2.7.1), DeepTools(v3.3.0), MACS(v2.2.6)