Small-molecule targeting of brachyury transcription factor addiction in chordoma

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Chordoma is a primary bone cancer with no approved therapy. The identification of therapeutic targets in this disease has been challenging due to the infrequent occurrence of clinically actionable somatic mutations in chordoma tumors. Here we describe the discovery of therapeutically targetable chordoma dependencies via genome-scale CRISPR-Cas9 screening and focused small-molecule sensitivity profiling. These systematic approaches reveal that the developmental transcription factor T (brachyury; TBXT) is the top selectively essential gene in chordoma, and that transcriptional cyclin-dependent kinase (CDK) inhibitors targeting CDK7/12/13 and CDK9 potently suppress chordoma cell proliferation. In other cancer types, transcriptional CDK inhibitors have been observed to downregulate highly expressed, enhancer-associated oncogenic transcription factors. In chordoma, we find that T is associated with a 1.5-Mb region containing 'super-enhancers' and is the most highly expressed super-enhancer-associated transcription factor. Notably, transcriptional CDK inhibition leads to preferential and concentration-dependent downregulation of cellular brachyury protein levels in all models tested. In vivo, CDK7/12/13-inhibitor treatment substantially reduces tumor growth. Together, these data demonstrate small-molecule targeting of brachyury transcription factor addiction in chordoma, identify a mechanism of T gene regulation that underlies this therapeutic strategy, and provide a blueprint for applying systematic genetic and chemical screening approaches to discover vulnerabilities in genomically quiet cancers.

Chordoma is a primary bone cancer that typically occurs in the skull-base, mobile spine and sacrum. Chordoma often manifests as chordal remnants. Both cell types share high expression of the T-box-family transcription factor brachyury (gene symbol: T; TBXT), an essential regulator of notochord development whose overexpression is characteristic of chordoma. Beyond being a chordoma biomarker, brachyury appears to be critical for disease pathogenesis: germline T duplication confers susceptibility to familial chordoma, a single-nucleotide polymorphism (SNP) in T is associated with chordoma, some sporadic chordomas harbor somatic copy-number gains of T, and T silencing inhibits growth of chordoma models. Furthermore, brachyury is primarily expressed in the embryo and is absent from the majority of normal adult tissue. These findings suggest that brachyury may act as an aberrantly activated developmental transcription factor that is oncogenic and essential in a lineage-specific manner, akin to canonical 'lineage-survival' oncogenes (for example, MITF in melanoma).

Notably, however, the full range of tumor dependencies in chordoma is not known. Few genes are recurrently mutated—and only at a modest frequency—in sporadic chordomas and nearly half of sporadic cases have no known driver mutation. Furthermore, no systematic functional genomics studies have been conducted in chordoma models. Thus, it remains unclear if brachyury represents the central tumor dependency of chordoma, or whether there are critical dependencies left to be uncovered and, if the former, whether brachyury overexpression can be targeted therapeutically. Similar to other transcription factors, brachyury is not readily inhibited pharmacologically and no small-molecule inhibitor of brachyury has been identified. It is also not known what underlies brachyury dysregulation in the majority of chordoma tumors, and whether any...

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potential mediators of overexpression are therapeutically targetable. Somatic alterations in T occur in a minority of sporadic chordomas and cannot explain the nearly universal occurrence of brachyury expression. Therefore, a deeper understanding of essential genes in chordoma, including potential regulators of brachyury expression, is imperative for nominating candidate therapeutic targets.

Recent advances in systematic CRISPR-Cas9 screening and small-molecule sensitivity profiling approaches have enabled identification of tumor dependencies in multiple cancer types. We integrated these complementary approaches to identify key tumor dependencies and candidate therapeutic targets in chordoma.

**T is a selectively essential gene in chordoma**

To identify genes essential for chordoma cell viability, we performed genome-scale pooled CRISPR-Cas9 loss-of-function screens in two chordoma cell lines (UM-Chor1, MUG-Chor1). We introduced a library of >74,000 single-guide RNAs (sgRNAs) targeting ~18,560 genes (Methods) into stably Cas9-expressing cells via lentiviral transduction and, after 21 d, quantified sgRNAs from the genomic DNA (gDNA) of surviving cells. Depleted sgRNAs, representing candidate essential genes, were identified by comparing these sgRNA abundances to those of the screening library. We ranked all sgRNAs by how much they reduced viability in chordoma cells relative to 125 non-chordoma cancer cell lines screened using the same sgRNA library (Broad Institute Project Achilles; https://depmap.org/portal/achilles) and removing commonly essential genes to identify dependencies selective for chordoma.

The top three selectively lethal sgRNAs, out of ~70,000 sgRNAs analyzed, all targeted the T gene (Fig. 1a). We confirmed that three of four T-targeted sgRNAs screened were lethal to both chordoma cell lines, but not to 125 non-chordoma cancer cell lines (Fig. 1b and Supplementary Table 1). Targeting T in chordoma cells induced a similar degree of viability reduction as targeting commonly essential ribosomal subunit genes (RPL23, RPS11 and RPS19 (Fig. 1b, Extended Data Fig. 1, and Supplementary Table 1)), indicating that chordoma cells are both exquisitely and selectively sensitive to loss of brachyury expression.

We validated the screening results with two sgRNAs targeting independent sequences of T in three chordoma cell lines (UM-Chor1, MUG-Chor1, U-CH2) (Fig. 1c). The observed reduction in chordoma cell proliferation induced by sgRNA-mediated brachyury repression was consistent with previously reported effects of sgRNA-mediated gene silencing of T in other chordoma cell lines.

SgRNA-mediated T repression in UM-Chor1 cells led to downregulation of canonical notochord markers (SOX9, COL2A1, ACAN) (Fig. 1d), consistent with brachyury's known role in notochord development. In addition, genes associated with the G2/M checkpoint and E2F targets were enriched among downregulated genes (Fig. 1e), suggestive of cell cycle arrest in sg-T-expressing cells. These data implicate T as an essential gene and regulator of notochord cell identity in chordoma.

### Inhibitors of CDK7/12/13 and CDK9 suppress chordoma cell proliferation

In parallel to the genetic screens, we tested 459 small molecules for their ability to reduce proliferation or survival in four chordoma cell lines. Compounds were selected to collectively target many distinct nodes in cell circuitry and included drugs approved by the Food and Drug Administration, preclinical agents, and small-molecule probes (Supplementary Table 2). A largely overlapping small-molecule library was previously tested in ~800 non-chordoma cancer cell lines, and we used these data to identify and prioritize compounds with antiproliferative effects selective for chordoma when possible (Fig. 2a and Methods).

Our analysis identified 28 potent antiproliferative compounds, including several inhibitors of CDK7/12/13, CDK9, or EGFR/ERBB2 proteins (Fig. 2a and Supplementary Table 2). Given that CDK7 and CDK9 have related roles in regulating transcription, we hypothesized that these inhibitor classes could have similar mechanistic effects.

The sensitivity of chordoma cells to compounds targeting CDK7/12/13 (THZ1), CDK12/13 (THZ314), CDK9 (NVP-A), and EGFR and/or ERBB2 (erlotinib, neratinib, canertinib, lapatinib, afatinib) was validated using multipoint concentration-response assays (Fig. 2b, Extended Data Fig. 2a, and Supplementary Table 3). Transcriptional CDK inhibitors including THZ1, dinaciclib, and alvocidib exhibit antiproliferative effects across a diversity of cancer cell lines (Extended Data Fig. 2b). Consistent with small-molecule sensitivity findings, sgRNAs targeting CDK7, CDK13, and CDK9, but not those targeting CDK12, were lethal to chordoma cells (Fig. 2c and Supplementary Table 1). sgRNAs targeting EGFR and ERBB2 were lethal to 2/2 and 1/2 chordoma cell lines, respectively (Fig. 2c and Supplementary Table 1). EGFR-inhibitor sensitivity in chordoma has been described previously; thus, we focused on transcriptional CDK inhibitors, which are newly identified antiproliferative agents in chordoma.

We confirmed on-target activity of the CDK7/12/13 inhibitor THZ1 by detecting reduced phosphorylation, particularly at serine 2, of the C-terminal domain (CTD) of RNA polymerase II (POLR2A) (Fig. 2d). Beyond having antiproliferative effects, THZ1 treatment induced apoptosis in chordoma cells, as measured by increased caspase-3/7 activity (Fig. 2e). We did not observe comparable sensitivity of chordoma cells to inhibitors of CDK proteins not implicated in direct phosphorylation of the POLR2A CTD, including CDK4/6 inhibitors (palbociclib/PD 0332991, LEE011) and CDK8/19 inhibitors (CCT251545, BRD6989) (Extended Data Fig. 3a and Supplementary Table 3). On-target activity was confirmed for a select number of CDK inhibitors tested (Extended Data Fig. 3b). Together, these data implicate inhibitors of CDK7/12/13 and CDK9 as potent antiproliferative agents in chordoma.

**T is super-enhancer-associated in chordoma**

Transcriptional CDK inhibitors are under clinical investigation (NCT03134638) owing to their ability to downregulate preferentially oncogenic transcription factors in multiple cancer types. Components of the transcriptional apparatus, including CDK7, can densely occupy super-enhancers—large clustered enhancers that drive genes required for tumor identity—often rendering the expression of super-enhancer-driven genes especially vulnerable to transcriptional inhibitors. To investigate whether similar mechanisms connect the essentiality of T to the antiproliferative effects of transcriptional CDK inhibitors, we tested whether T is super-enhancer-associated in chordoma.

We mapped the enhancer landscape in five chordoma cell lines using chromatin immunoprecipitation sequencing (ChIP-seq) to detect histone H3 lysine 27 acetylation (H3K27ac), a chromatin modification associated with active enhancers. We identified large enhancers overlapping and adjacent to the T locus in all five models (Fig. 3a, Extended Data Fig. 4, and Supplementary Table 4). T enhancers showed evidence of brachyury binding, as measured by brachyury ChIP-seq (using a published dataset) (Fig. 3a), and potentially other transcription factors as suggested by an assay for transposable-accessible chromatin using sequencing (ATAC-seq) (Fig. 3a), which measures chromatin accessibility and transcription factor occupancy. In contrast, super-enhancers and brachyury binding were not detected at the housekeeping transcription factor MAX locus, despite the presence of other transcription factor binding sites, as inferred by ATAC-seq signal (Fig. 3b and Supplementary Table 4).

All chordoma cell lines had high T (brachyury) expression, especially compared with non-super-enhancer-associated genes, such as
**Fig. 1** | Genome-scale CRISPR-Cas9 screening identifies T (brachyury) as a selectively essential gene in chordoma cells. 

**a.** Comparative analysis of genome-scale CRISPR-Cas9 screening results generated in UM-Chor1 and MUG-Chor1 chordoma cell lines versus 125 non-chordoma cancer cell lines. SgRNAs were ranked by and plotted against selectively lethal effects (RNMI scores; see Methods) in chordoma versus non-chordoma cell lines. The top three sgRNAs (red circles) all target T. b. Viability after sgRNA treatment (represented by sgRNA-dependency scores; see Methods) corresponding to each of the four primary screening sgRNAs targeting either T or RPL23 across 127 cancer cell lines (CCLs), including two chordoma CCLs (blue circles). For each sgRNA, the median sgRNA-dependency score across 127 cell lines is indicated (gray line). Lower values indicate greater sgRNA depletion and thus essentiality of the target gene (shaded region). c. (Left) Proliferation of chordoma cell lines transduced with sgRNAs targeting T or a non-targeting sgRNA control. Points represent the mean ± s.d. (n = 3 biological samples measured in parallel). **** P < 0.0001, derived from a two-way analysis of variance (ANOVA) (P values for the test comparing sg-EGFP and sg-T_1 are displayed). Exact P values and effect sizes are reported in Supplementary Table 9. (Right) Immunoblot analysis of transduced cells confirming sgRNA-mediated protein repression. Proliferation experiments were performed twice with UM-Chor1 and U-CH2 cells (one representative experiment displayed for each) and once with MUG-Chor1 cells; immunoblots were performed once. d. Relative gene expression of UM-Chor1 cells transduced with one of two sgRNAs targeting T versus a non-targeting sgRNA control. Gene expression was measured with RNA sequencing. Data represent two biological replicates per condition. e. Gene-set enrichment analysis results show significant downregulation of genes associated with cell cycle progression, such as drivers of G2/M checkpoint progression and E2F targets.
Fig. 2 | Small-molecule sensitivity profiling identifies inhibitors of CDK7/12/13 and CDK9 as potent antiproliferative agents in chordoma cells.

a, Profiling results for 459 small molecules tested in four chordoma cell lines. Data represent area-under-curve (AUC) values calculated from eight-point concentration-response curves generated in duplicate, and coloring in the heatmap is representative of smaller AUC values (blue) corresponding to more potent effects (AUC value of 1 = vehicle control; value of 0 = complete killing at all concentrations). Left heatmap, all compounds were ranked by average potency in chordoma cell lines; potent compounds (average AUC < 0.8, boxed) were then filtered to exclude those with cytotoxic effects non-selective for chordoma cell lines versus up to 891 non-chordoma cancer cell lines, when such data were available in the Cancer Therapeutics Response Portal (CTRP). Compounds that were only tested in chordoma cell lines and were not available in CTRP were not filtered beyond the initial potency filter. Twenty-eight compounds (right heatmap) passed these criteria. b, Validation of primary screening hits and related compounds. Four chordoma cell lines were treated with indicated concentrations of candidate antiproliferative compounds and assayed for cell viability after 6 d with CellTiter-Glo. Response data are represented by a fitted curve to the mean fractional viability at each concentration relative to vehicle-treated cells; error bars represent the s.e.m. (n = 4 biological samples measured in parallel). c, Rankings of sgRNA-level dependency scores (see Methods) for the indicated genes following genome-scale CRISPR-Cas9 screening (see Fig. 1). Points represent each of four sgRNAs targeting a given gene that were present in the pooled CRISPR library used for screening. P values were derived from a one-sided Mann–Whitney test. *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant. The gray waterfall plot represents ranked dependency scores for all sgRNAs tested (median dependency score = −0.3257 for MUG-Chor1; median = −0.3283 for UM-Chor1). Median dependency scores for the four sgRNAs and exact Mann–Whitney P values corresponding to each gene are reported in Supplementary Table 9. d, Immunoblot analysis of UM-Chor1 cells treated with indicated concentrations of THZ1 or DMSO for 24 h. Data are representative of two independent experiments. e, Caspase-3/7 activity (top) and cell viability (bottom) following THZ1 treatment of UM-Chor1 cells. Caspase-3/7 activity and cell viability were measured in parallel at the indicated time points using Caspase-Glo 3/7 and CellTiter-Glo reagents, respectively. Data are expressed as the fold change of caspase-3/7 activity (top) or fraction of cell viability (bottom) relative to vehicle-treated cells and represent the mean ± s.d. (n = 4 biological samples measured in parallel).
Fig. 3 | T (brachyury) is super-enhancer-associated and highly active across chordoma cell lines and in patient-derived chordoma tumors. **a**, **b**, Gene tracks of H3K27ac, brachyury, and ATAC-seq signal (units of reads per million per base pair) at the T and MAX gene loci. For datasets with multiple samples (H3K27ac and ATAC-seq), signals for samples are plotted as a translucent shape and darker regions indicate regions with signal in more samples. An opaque line is plotted along the vertical axis and were determined by the inflection point of the plot. **e**, **f**, Gene expression levels of the top 30 (of 115) super-enhancer (SE)-associated transcription factors (TFs) in patient-derived chordoma MGH_1 chordoma tissue (4 MGH and 4 CF samples). **c**, **d**, Enhancers in chordoma cell lines (UM-Chor1, MUG-Chor1, U-CH2, U-CH1, JHC7); brachyury ChIP-seq was performed on one chordoma cell line (U-CH1) as previously reported and ATAC-seq was performed on two chordoma cell lines. **g**, **h**, Patient-derived chordoma MGH_1. **i**, **j**, Clustering of chordoma H3K27ac SEs.
Transcriptional CDK inhibition downregulates T (brachyury) expression preferentially

The essentiality of T, together with its association with super-enhancers, led us to test whether the antiproliferative effects of transcriptional CDK inhibitors are mediated by preferential loss of brachyury expression.

We found that THZ1 treatment reduced cellular brachyury protein levels in MUG-Chor1 and UM-Chor1 cells in a concentration- and time-dependent manner (Fig. 4a and Extended Data Fig. 8a). In contrast, brachyury protein expression was unchanged following treatment with compounds that are cytotoxic to chordoma cells, but are not transcriptional CDK inhibitors (Supplementary Table 2), including inhibitors of EGFR (erlotinib), Src (dasatinib), and the BET bromodomain (JQ1) (Fig. 4a). Downregulation of brachyury expression was also observed following treatment with CDK9 inhibitors (NVP-2, dinaciclib, alvocidib), but not with the CDK4/6 inhibitor palbociclib, across multiple chordoma models (Fig. 4b).

THZ1 treatment also substantially reduced mRNA expression of T, especially compared with MAX or the housekeeping gene CFL1 (Extended Data Fig. 8b). For comparison, we tested actinomycin D, a transcriptional inhibitor not thought to act through CDK inhibition 1, and similarly observed mRNA downregulation of T, but not MAX or CFL1 (Extended Data Fig. 8b). However, compared with THZ1, actinomycin D induced higher cytotoxicity at concentrations leading to substantial T-mRNA downregulation (Extended Data Fig. 8b,c and Supplementary Table 3), suggesting that THZ1 and actinomycin D could differ in how selectively they induce T downregulation.

We confirmed this difference using transcriptome-wide gene-expression profiling of THZ1- and actinomycin-D-treated cells (Fig. 4c). Only THZ1 treatment led to preferential downregulation of T expression (Fig. 4c), indicating that super-enhancer-driven T transcription is particularly vulnerable to CDK7/12/13 inhibition.

Furthermore, we tested whether THZ1’s antiproliferative effects are mediated by loss of super-enhancer-driven brachyury expression. To dissociate T from its endogenous regulatory elements, we transduced UM-Chor1 cells with an ORF encoding T under the control of an exogenous promoter. Unlike endogenous brachyury protein, ectopic brachyury expression levels were not greatly reduced with THZ1 treatment (Fig. 4d). Moreover, ectopic brachyury expression was sufficient to partially rescue loss of viability induced by 500 nM THZ1 treatment (Fig. 4e). Thus, downregulation of brachyury via its endogenous regulatory elements directly contributes to the antiproliferative effects of THZ1.

Besides T, only four genes were identified to be both super-enhancer-associated and essential in UM-Chor1 and MUG-Chor1 cells: SOX9, SAE1, TPX2, and ATP6V1B2 (Fig. 4f). These genes appear to be under direct and/or indirect transcriptional control by brachyury: sgRNA-mediated T repression significantly reduced expression levels of all four genes (Fig. 4f, Supplementary Table 8, and Extended Data Fig. 9) and brachyury bound genomic regions corresponding to two of them (SAE1 and ATP6V1B2; Fig. 4f and Supplementary Table 8).

These data support a model whereby T (brachyury) is expressed at high levels by a super-enhancer, is auto-regulated, and, through direct binding to other super-enhancers and/or indirect mechanisms, can regulate other essential, super-enhancer-associated genes (Fig. 4g). As transcriptional CDKs including CDK7 and CDK9 are localized at enhancers4,31, these data implicate transcriptional CDK inhibition in both upstream and downstream targeting of T (brachyury) gene control.

Finally, THZ1 efficacy was tested in vivo. A xenograft mouse model was generated with the human chordoma cell line CH22 1. Ex vivo, CH22 cells were sensitive to transcriptional CDK inhibitors (Extended Data Fig. 10a), and compound treatment induced down-regulation of brachyury expression (Extended Data Fig. 10b). Mice were treated with 40 mg kg⁻¹ THZ1 BID, on a pulse-dosing schedule (Fig. 4h). This dose was reasonably well tolerated (Extended Data Fig. 10c), and was empirically determined to downregulate brachyury expression in target engagement studies of mouse tumors (Extended Data Fig. 10d), although we note that detection of brachyury downregulation varied across experiments and mice (Extended Data Fig. 10e).

THZ1 treatment led to a significant tumor-suppressive effect (P = 1.1 × 10⁻⁵, Fig. 4i and Supplementary Table 9). These data provide evidence for the in vivo efficacy of THZ1 for the treatment of chordoma.

In conclusion, these findings nominate transcriptional CDK inhibition as a therapeutic strategy to target brachyury-driven transcriptional addiction in chordoma. Our results indicate that T (brachyury), a transcriptional regulator of notochord development, might drive chordoma tumorigenesis when dysregulated...
or persistently expressed. Thus, \( T \) resembles other ‘lineage-survival’ oncogenes (for example, \( MITF \) in melanoma), which arise from dysregulated expression of master genes that mediate normal lineage development \(^7\). More generally, these findings provide evidence for ‘transcriptional addiction’ in chordoma, a state of exquisite dependence on specific regulators of gene expression \(^5\). As transcriptional dependencies are not typically discovered by tumor genome sequencing, these findings underscore the value of functional screening approaches, such as CRISPR-Cas9 and small-molecule sensitivity screening, to identify key vulnerabilities that may not manifest as mutations in the tumor genome.

Transcription factors, including brachyury, are challenging to target directly with candidate drugs. Transcriptional CDK inhibition provides a therapeutic opportunity to downregulate preferentially...
T (brachyury). The recent emergence of next-generation transcriptional CDK inhibitors into the clinic may provide a much-needed therapeutic option for chordoma patients with refractory disease.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41591-018-0312-3.

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References

1. Stacchiotti, S., Sommer, J. & Chordoma Global Consensus Group. Building a global consensus approach to chordoma: a position paper from the medical and patient community. Lancet. Oncol. 16, e71–e83 (2015).
2. Choy, E. et al. Genotyping cancer-associated genes in chordoma identifies mutations in oncogenes and areas of chromosomal loss involving CDKN2A, PTEN, and SMARCB1. PLoS ONE 9, e101283 (2014).
3. Tarpney, P. S. et al. The driver landscape of sporadic chordoma. Nat. Commun. 8, 890 (2017).
4. Kwiatkowski, N. et al. Targeting transcription regulation in cancer with a molecule sensitivity dataset. Mol. Cell 60, 328–337 (2015).
5. McMaster, M. L., Goldstein, A. M., Bromley, C. M., Ishibe, N. & Parry, D. M. Brachyury: a crucial regulator of notochordal development, is a novel biomarker for chordomas. J. Pathol. 268, 629–643 (2017).
6. Showell, C., Binder, O. & Conlon, F. L. T-box genes in early embryogenesis. Nat. Rev. Cancer 13, 115–128 (2013).
7. Barry, J. J. et al. The next step: innovative molecular targeted therapies for treatment of intracranial chordoma patients. Neurosurgery 68, 231–240 (2011). Discussion 240–231.
8. Salisbury, J. R. The pathology of the human notochord. J. Pathol. 171, 253–255 (1993).
9. Vujovic, S. et al. Brachury, a crucial regulator of notochordal development, is a novel biomarker for chordomas. J. Pathol. 209, 1557–1567 (2006).
10. Showell, C., Binder, O. & Conlon, F. L. T-box genes in early embryogenesis. Dev. Dyn. 229, 201–218 (2004).
11. Yang, X. R. et al. T (brachyury) gene duplication confers major susceptibility to familial chordoma. Nat. Genet. 41, 1176–1178 (2009).
12. Pillay, N. et al. A common single-nucleotide variant in T is strongly associated with chordoma. Nat. Genet. 44, 1185–1187 (2012).
13. Presneau, N. et al. Role of the transcription factor T (brachyury) in the pathogenesis of sporadic chordoma: a genetic and functional-based study. J. Pathol. 223, 327–335 (2011).
14. Hsu, W. et al. Generation of chordoma cell line JHC7 and the identification of novel molecular target. J. Neurosurg. 115, 760–769 (2011).
15. Shah, S. R. et al. Brachury-YAP regulatory axis drives stenosis and growth in chordoma. Cell Rep. 21, 495–507 (2017).
16. Palena, C. et al. The human T-box mesodermal transcription factor Brachury is a candidate target for T-cell-mediated cancer immunotherapy. Clin. Cancer Res. 13, 2471–2478 (2007).
17. Garraway, L. A. & Sellers, W. R. Lineage dependency and lineage-survival oncogenes in human cancer. Nat. Rev. Cancer 6, 593–602 (2006).
18. Berg, T. Inhibition of transcription factors with small organic molecules. Curr. Opin. Chem. Biol. 12, 464–471 (2008).
19. Sharafiua, T., Hong, A. L., Painter, C. A. & Boehm, J. S. Emerging opportunities for target discovery in rare cancers. Cell Chem. Biol. 24, 1075–1091 (2017).
20. Meyers, R. M. et al. Computational correction of copy number effect improves specificity of CRISPR-Cas9 essentiality screens in cancer cells. Nat. Genet. 49, 1779–1784 (2017).
21. Barriouneau, F., Taketo, M. M., Scherer, G. & Kispert, A. Sox9 is required for notochordal maintenance in mice. Dev. Biol. 295, 128–140 (2006).
22. Seashore-Ludlow, B. et al. Harnessing connectivity in a large-scale small-molecule sensitivity dataset. Cancer Discov. 5, 1210–1223 (2015).
23. Whittaker, S. R., Mallinger, A., Workman, P. & Clarke, P. A. Inhibitors of cyclin-dependent kinases as cancer therapeutics. Pharmacol. Ther. 173, 83–105 (2015).
24. Zhang, T. et al. Covalent targeting of remote cysteine residues to develop CDK12 and CDK13 inhibitors. Nat. Chem. Biol. 12, 876–884 (2016).
25. Galli, G. G. et al. YAP drives growth by controlling transcriptional pause release from dynamic enhancers. Mol. Cell 60, 328–337 (2015).
26. Modjtahedi, H., Cho, B. C., Michel, M. C. & Solca, F. A comprehensive review of the preclinical efficacy profile of the ErbB family blocker afatinib in cancer. Naunyn Schmiedebergs Arch. Pharmacol. 387, 505–521 (2014).
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**Additional information**

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The Mann–Whitney test reported in Fig. 2c was performed in MATLAB (MathWorks, Inc.).

Validation of CRISPR-Cas9 screens. UM-Chor1, MUG-Chor1 and U-CH2 cells were each incubated with lentivirus corresponding to the pXPm_001 plasmid, encoding sg-T_1, sg-T_2 or sg-EGFP (sequences below), in the presence of 4 μg/ml polybrene, dispensed in 12-well plates (1.5 × 10^6 cells per well for UM-Chor1 and MUG-Chor1, 1.2 × 10^6 cells per well for U-CH2) and spin-infected at 2,000 r.p.m. for 2 h at 30 °C. After spin-infection, 2 ml of standard growth media was added to each well and cells were incubated at 37 °C overnight. The following day, for each cell line, cells were trypsinized and expanded in selective media containing puromycin (3 μg/ml for UM-Chor1, 1–2 μg/ml for MUG-Chor1, 3 μg/ml for U-CH2). Following selection for infected cells, cells were seeded in 24-well plates in selective media (30,000 cells per well for UM-Chor1 and U-CH2 and 23,000 cells per well for MUG-Chor1, with three replicates per condition), and counted at indicated intervals over a 25 d period. In parallel, selected cells were collected (at least 9 d post-transduction) for immunoblotting to confirm sgRNA-mediated protein reduction. Proliferation experiments were performed twice for UM-Chor1 and U-CH2 with minor modifications between experiments and once for MUG-Chor1, with three replicates per experiment. Immunoblots were performed once. Statistical analysis was done using GraphPad Prism v.7.

Lentiviral vectors used for screening validation and functional characterization. The 7-targeting sgRNAs used for low-throughput experiments were cloned in the pXPm_001 lentiviral vector, which encodes the Cas9 protein. Spacer sequences for the following: sg-T_1, CCCTGAGACCCAGTTCATAG; sg-T_2, TGGCTGTGGTGATCGCCTG. The pXPm_001 plasmid encoding sg-EGFP was provided by the laboratory of E. Zhang (Broad Institute) and has been described previously (“EGFP sgRNA 6”). Lentivirus was produced by transfection of 293 T packaging cells with three plasmids (pXPm_001-sgRNA, Δ8.9 [gag, pol], and pMD2.G); and the FuGene6 transfection reagent (Roche). Virus-containing supernatant was collected 3 d post-transfection.

Small-molecule sensitivity profiling. Chemical sensitivity tests similar to those reported in the CTRP (www.broadinstitute.org/ctrp/) were performed using 459 small molecules chosen collectively to target a diverse set of nodes in cell circuitry and with a high degree of overlap to published informer sets (Supplementary Table 2), including 428 compounds published in the CTRP. UM-Chor1, MUG-Chor1, U-CH1 and JHC7 cells were each seeded overnight in 384-well BioCoat Collagen I (Corning) microtitre plates at a density of 1,200, 1,800, 2,000 and 1,600 cells per well, respectively. The following day, compound or DMSO was added to wells (1,400 dilution) using a CyBio pinning instrument. Each compound was tested using eight concentrations in duplicate. Cells were incubated at 37 °C, and cell viability was assayed 6 d after the addition of compound or DMSO using the CellTiter-Fluor reagent (Promega). For each cell line, primary screening was performed once with two replicates.

Computational analysis software for small-molecule sensitivity studies. Computational analyses and visualizations were performed in Microsoft Excel Professional Plus 2013, GraphPad Prism (v.6 or v.7), Pipeline Pilot (v.8.5) (Accelyrs, Inc.), or MATLAB 8.4 (2014b) or MATLAB 9.4 (2018a) (MathWorks, Inc.), as described in the following specific Methods sections.

Identification of candidate antiproliferative small molecules in chordoma.

The response of each chordoma cell line to a tested compound was quantified as follows: log-transformed duplicate data were averaged during normalization of luminescence values to vehicle (DMSO) treatment and, at each compound concentration, an average percentage-viability score was calculated. Percentage-viability response-point measurements were computed across all cell lines, compounds and concentrations. Curves were fit with nonlinear sigmoid functions, and the AUC for each CCL–compound pair was calculated by numerically integrating under the eight-point concentration–response curve essentially as described previously. In our analysis, we differed from the published procedure in (1) the number of concentrations tested; (2) the use of fixed concentration limits of integration for AUCs across all compounds; (3) standardization of AUC values on the range from 1 (DMSO or no effect) to 0 (complete killing throughout the concentration range of integration); see https://github.com/remontone-pac/ctrp-reference/tree/master/auc. Data pre-processing from instrument files through DMSO normalization was performed in Pipeline Pilot, and curve-fitting, numerical integration and subsequent analysis steps were performed in MATLAB.

To identify candidate antiproliferative small molecules in chordoma, a threshold was applied to include only potent compounds (average AUC across four chordoma cell lines <0.8). Next, to assess whether a compound had selectively cytotoxic effects in chordoma lines relative to other cell lines tested in the CTRP, we computed the median AUC for four chordoma lines and z-scored this median with respect to the mean and standard deviation of all other cell lines tested with that compound. We applied this selectivity analysis to filter out potent compounds whose cytotoxic effects were non-selective for chordoma cell lines (z < −1), when those data were available. We note that some compounds were only tested in the
chordoma lines, and therefore data about their performance across CTRP lines were not available; in such cases, we did not apply any additional filtering step beyond the initial potency filter. Twenty-eight compounds passed these criteria and 43 compounds were filtered out. Compounds targeting proteins of interest (CDK7/12/13/9 and EGFR/ERBB2) were carried forward for secondary screening.

Validation of small-molecule sensitivity screens. UM-Chor1, MUG-Chor1, U-Ch1, JHC7 and CH22 cells were each seeded overnight in 384-well BioCoat Collagen I (Corning) microtiter plates at a density of 1,200, 1,800, 2,000, 1,600, and 1,200 cells per well, respectively. The following day, compound or DMSO was added to wells using an HP D300 digital dispenser instrument. Each compound was tested using nine concentrations, in quadruplicate (four wells treated in parallel). Cell viability was assayed 6 d after compound addition with the CellTiter-Glo reagent (Promega). Luminescence values were treated as with the previous experiment (see previous Data preprocessing from instrument files through DMSO normalization was performed in Pipeline Pilot for UM-Chor1, MUG-Chor1, U-Ch1, JHC7 or Microsoft Excel for CH22; and curve-fitting, numerical integration and subsequent analysis steps were performed in MATLAB. Experiments depicted in Fig. 2b and Extended Data Figs. 2a and 3a were performed at least twice in each cell line (except atabmix treatment in JHC7 cells, which was performed once); experiments depicted in Extended Data Fig. 8c were performed at least three times in UM-Chor1 cells and once for all other cell lines. Experiments depicted in Extended Fig. 10a were performed once. For experiments performed multiple times, compound addition was performed using either a digital dispenser instrument, as described above, or a Cytobio pinning instrument.

Small-molecule reagents. Small molecules used for low-throughput ex vivo experiments were obtained from the following sources: THZ1, THZ531 and NVP-2 were provided by the laboratory of N. Gray (Dana-Farber Cancer Institute); Q11 was provided by the laboratory of J. Bradner (Dana-Farber Cancer Institute). THZ531 and NVP-2 was provided by TAE (The Institute of Cancer Research); alvocidib, AT7519, dinaciclib, LEE011 and palbociclib were purchased from Selleck Chemicals; erlotinib and dasatinib were purchased from LC Laboratories; actinomycin D was purchased from G1bio; neratinib, canertinib, lapatinib and afatinib were obtained from Broad Institute (The Institute of Cancer Research); CCT251545 was provided by P. Clarke and A. Mallinger (The Institute of Cancer Research); THZ1 Hydrochloride used for in vivo studies was obtained from MedChemExpress.

RNA sequencing. For RNA sequencing experiments with sgRNA-treated cells: UM-Chor1 cells were incubated with lentivirus corresponding to the pXPR_001 plasmid, encoding sg-T_1, sg-T_2 or sg-EGFP in the presence of 4 μg ml⁻¹ polybrene, dispensed in 12-well plates (1.3 x 10⁶ cells per well) and spin-infected at 2,000 r.p.m. for 2 h at 30 °C. After spin-infection, 2 ml of standard growth media was added to each well and cells were incubated at 37 °C overnight. The following day, cells were trypsinized and expanded in selective media containing puromycin (2 μg ml⁻¹). Following selection for infected cells, selective media was replaced with standard growth media and cells were collected 2 d later. Cell pellets were used for RNA extraction using an RNeasy Kit (Qiagen). Sequencing libraries were prepared according to the manufacturer’s instructions for relative quantity calculations using the Delta Ct method. For each sample, cycle threshold (Ct) values were averaged across technical replicates. The ΔCt values were then calculated by subtracting the average reference-gene (GAPDH) Ct values from average target-gene (Ct) values, for each biological replicate. We applied a one-sided Welch’s t-test comparing ΔCt values for treatment and reference samples to determine significant differences. Log₂-fold-changes were calculated as ΔCt values by subtracting ΔCt values for the reference samples from ΔCt values for the treated samples. All calculations were done in R (3.4.4).

Caspase-3/7 activity assay. UM-Chor1 cells were seeded overnight in 384-well BioCoat Collagen I (Corning) microtiter plates at a density of 1,200 cells per well. The following day, media was replaced with standard growth media containing compound at the final concentration or DMSO. Compound- and DMSO-treated cells were collected 4 h after compound or DMSO addition. Cell pellets were used for RNA extraction (Qiagen). Sequencing libraries were prepared using the TruSeq Stranded mRNA Library Prep Kit (Illumina) and libraries were sequenced using the Illumina HiSeq 2500 instrument (rapid-run mode) with paired-end 100 base pair (bp) reads. Sequencing was performed once with two biological replicates for each condition.

RNA sequencing analysis. For RNA-sequencing experiments with compound-treated cells: UM-Chor1 cells were seeded overnight in 6-cm collagen I-coated dishes at a density of 750,000 cells per dish. The following day, media was replaced with standard growth media containing compound at the final concentration or DMSO. Compound- and DMSO-treated cells were collected 4 h after compound or DMSO addition. Cell pellets were used for RNA extraction (Qiagen). Sequencing libraries were prepared using the TruSeq Stranded mRNA Library Prep Kit (Illumina) and libraries were sequenced using the Illumina NextSeq 500 instrument with single-end, 75bp reads. Sequencing was performed once with two biological replicates for each condition.

RNA-seq screening. For all RNA-seq experiments, raw reads were processed with Trim Galore! (v.0.4.1) (Babraham Bioinformatics; https://www.bioinformatics. babraham.ac.uk/projects/trim_galore/) to remove adapter sequences and aligned with STAR (v.2.4.2j) to the human genome (GRCh38, primary assembly) using the GENCODE annotation (v.23). Differential-expression analysis was done using R (v.3.2.3) (www.r-project.org/) using the DESeq2 package (v.1.10.0). Gene set enrichment analysis was done using GSEA2 (v.2.0.0) and gene sets from MSigDB (v.5.0) [32]. We used the ‘preranked’ algorithm to analyze gene lists ranked by the negative decadic logarithm of adjusted P values obtained from the differential-expression analysis with DESeq2. To separate up- and downregulated genes, we artificially assigned a negative sign to values for downregulated genes (thus using the decadic logarithm of adjusted P values). We used the options – nperm 1,000, –set_max 1,500, and –set_min 5.

Copy number variation analysis. Whole-exome sequencing data for all chordoma cell lines were obtained from the Chordoma Foundation (available at www.cavatica.org) and processed according to the GATK Best Practices [33]. Briefly, starting with reads in an unaligned BAM file, we marked sequencing adapters with Picard (v.1.999) MarkIlluminaAdapters, aligned reads to the human genome (hg19) with BWA (v.0.7.12), and marked duplicates with Picard MarkDuplicates (Broad Institute Picard; https://github.com/broadinstitute/picard). We then used the GATK3 toolkit (v.2.2) to perform Indel Realignment and Base Recalibration (Broad Institute GATK; software.broadinstitute.org/gatk) [34]. Somatic copy-number variations were determined using the GATK4 toolkit (Broad Institute GATK; https://gatkforums.broadinstitute.org/gatk/discussion/9143/how-to-call-somatic-copy-number-variants-using-gatk4-cnv).

RT-qPCR. RT-qPCR of compound-treated cells. UM-Chor1 cells were seeded overnight in six-well collagen I-coated dishes at a density of 275,000 cells per well. The following day, media was replaced with standard growth media containing compound at the final concentration or DMSO. Compound- and DMSO-treated cells were collected 4, 8 and 12 h after compound or DMSO addition, and cell pellets were used for RNA extraction using an RNeasy Kit (Qiagen). Three biological replicates were generated per condition. Total RNA was reverse transcribed to cDNA using the High-Capacity RNA to-cDNA Kit (Applied Biosystems); cDNA was used for qPCR to analyze the TAP7 Gene Expression Master Mix and Taqman Gene Expression probes (Applied Biosystems). For each cDNA sample, four technical replicate reactions were performed using probes for the gene of interest and, in parallel, for the GAPDH reference gene. The Taqman probes used in the study were as follows: T, hs006010880_m1; MAX, hs00811069_g1; CFL1, hs02621564_g1; GAPDH, hs02758991_g1. Amplification was performed using the StepOne Plus 2.0 (Applied Biosystems). The experiment was performed twice with variations in cell seeding density and replicate numbers, with similar conclusions.

RT-qPCR of sgRNA-treated cells. UM-Chor1 cells were transduced with sgRNAs targeting T and EGFP and subjected to RNA extraction as described in the RNA-sequencing methods section. Total RNA was used for cDNA synthesis and qPCR, with four technical replicates per qPCR reaction condition, as described above. The Taqman probes used in the study were as follows: T, hs00610080_m1; GAPDH, hs02758991_g1; ATP6V1B2, hs00156037_m1; SAE1, hs00271440_m1; SOX9, hs00165814_m1; TFX2, hs00201616_m1. The experiment was performed once with two biological replicates for each sgRNA.

Analysis of RT-qPCR data. RT-qPCR data were analyzed according to the instrument manufacturer’s instructions for relative quantity calculations using the ΔΔCt method. For each sample, cycle threshold (Ct) values were averaged across technical replicates. The ΔCt values were then calculated by subtracting the average reference-gene (GAPDH) Ct values from average target-gene (Ct) values, for each biological replicate. We applied a one-sided Welch’s t-test comparing ΔCt values for treatment and reference samples to determine significant differences. Log₂-fold-changes were calculated as ΔΔCt values by subtracting ΔCt values for the reference samples from ΔΔCt values for the treated samples. All calculations were done in R (3.4.4).

Compound or sgRNA treatment for immunoblot analysis. For Fig. 4a: MUG-Chor1 and UM-Chor1 cells were seeded overnight in 6-cm collagen I-coated dishes at a density of 750,000 cells per dish. The following day, media was replaced with standard growth media containing compound at the final concentration or DMSO. Cells were collected 48 h after compound or DMSO addition and cell pellets were used for immunoblot analysis. The experiment was performed four times for UM-Chor1 with variations in compound concentration and incubation times, with similar conclusions and once for MUG-Chor1. For Fig. 4b using the Extended Data Fig. 10b: MUG-Chor1, UM-Chor1, U-Ch1, JHC7, and CH22 cells were each seeded overnight in 6-cm collagen I-coated dishes at a density of 750,000 cells per dish. The following day, media was replaced with standard growth media containing compound at the final concentration or DMSO. Cells were collected 48 h after compound or DMSO addition and cell pellets were used for immunoblot analysis. The experiment was performed twice for
alcohols as follows: 50% ethanol, 2 min; 70% ethanol, 2 min; 95% ethanol, 2 min; 100% ethanol, 2 min (×2) and xylene, 5 min (×2). Slides were mounted using Cytoseal (Richard-Allan Scientific).

Images were converted to high-resolution digital images using Leica ScanScope XT. Images were visualized for figures using Aperio ImageScope software.

ChIP. For H3K27ac ChIP of patient-derived tissue: approximately 100 mg of flash-frozen tissue was minced into 1–2-mm pieces and incubated in 1% formaldehyde for 15 min, followed by quenching with glycine (125 mM final concentration). Fixed tissue pieces were homogenized with a Tissue Tearer rotor stator homogenizer (Biospec) set to 30,000 r.p.m. for 60 s. Homogenate was washed with ice-cold PBS containing x1 HALT protease inhibitor. Homogenized pellets were then resuspended in cytosolic lysis buffer (50 mM HEPES pH 7.5, 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% IGEPAI, 0.25% Triton X-100, x1 HALT protease inhibitor) and the protocols indicated above for ChIP-seq were used.

For H3K27Ac ChIP of chordoma cell lines: Chordoma cell lines were grown in 175-cm2 collagen I-coated plates (three plates per cell line) and cross-linked by adding 1/10 of the cell culture volume of 11% formaldehyde solution (1 M HEPES-KOH pH 7.5, 0.5 M EDTA pH 8.0, 0.5 M EGTA pH 8.0, 5 M NaCl, 37% formaldehyde) for 10 min, followed by quenching (125 mM glycine). Cells were washed in PBS and collected by cell scraper in PBS. Cells were centrifuged at 1,350g for 5 min at 4 °C, washed with PBS, and centrifuged again at 1,350g for 5 min at 4 °C. Cell pellets were flash frozen and stored at −80 °C until further processing.

For both cell line and tissue ChIP-seq: samples were subsequently rotated end-over-end in nuclear lysis buffer for 1 h on ice, centrifuged at 1,350g for 5 min. Samples were resuspended in nuclear lysis buffer (10 mM Tris-HCl pH 8, 200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, x1 HALT protease inhibitor) and rotated end-over-end for 10 min at 4 °C and spun at 1,350g for 5 min. Samples were centrifuged in 1 ml sonication buffer (0.1% sodium deoxycholate, 50 mM HEPES pH 7.5, 140 mM NaCl, 1 mM EDTA, 1 mM EGTA, x1 Triton X-100, x1 HALT protease inhibitor) for a final concentration of 10% SDS. Supernatants were collected and the protein concentration was determined by sonication using a Bioruptor water bath sonicator (Diagenode) for 25 cycles. Samples were clarified by centrifuging 10 min at 20,000g at 4 °C. A 50-µl aliquot of supernatant was set aside as non-IP input control. The remaining sheared chromatin was diluted 1:5 in sonication buffer and each sample was incubated overnight at 4 °C with 100 µl of Protein G Dynabeads (Life Technologies) bound with 10 µg of anti-H3K27ac antibody (abcam, no. ab4729). Beads were washed while rotating end-over-end at 4 °C with the following buffers: twice in sonication buffer, once in sonication buffer with NaCl added to a final concentration of 500 mM, once in LiCl wash buffer (20 mM Tris pH 8, 1 mM EDTA, 250 mM LiCl, 0.5% IGEPAI, 0.5% sodium deoxycholate) and once in TE-NaCl buffer (50 mM Tris-HCl pH 8, 50 mM NaCl). Chromatin was eluted by resuspending beads in 200 µl elution buffer (50 mM Tris-HCl pH 8, 10 mM EDTA, 1% SDS) and heating for 15 min at 65 °C. Beads were pelleted by centrifuging at 20,000g for 30 s and supernatant was collected. Elution buffer (150 µl) was also added to input chromatin samples. Samples were incubated at 65 °C overnight to reverse crosslinks. Samples were then incubated with RNase A (Thermo Scientific) for 2 h at 37 °C followed by incubation with Proteinase K (Ambion) for 30 min at 55 °C. DNA isolation was performed via phenol–chloroform extraction and concentrated by ethanol precipitation. Sequencing libraries were generated with the TruSeq DNA Sample Single Index Kit (Rubicon) and sequenced on an Illumina NextSeq 500 with single-end 75 bp reads. ChIP-seq was performed once for each sample.

ATAC. For each cell line (MUG-Chor1 and U-CH2), 50,000 cells were lysed for 10 min at 4 °C in lysis buffer (10 mM Tris-HCl pH 7.4, 10 mM NaCl, 3 mM MgCl2, 0.1% IGEPAI CA-360). After lysis, the pellets were subject to a transposition reaction (37 °C, 60 min) using the x2 TD buffer and transposase enzyme (Illumina Nextera DNA preparation kit, FC 121-1030). The transposition mixture was purified using a Qagen MinElute PCR purification kit. Library amplification was performed using custom Nextera primers and the number of total cycles determined by running a SYBR-dye based qPCR reaction and calculating the cycle number that corresponds to a quarter of the maximum. Amplified libraries were then incubated with RNase A (Thermo Scientific) for 2 h at 37 °C followed by end-over-end 100 bp reads on an Illumina HiSeq 2000. ATAC-seq was performed once for each cell line, with two biological replicates for U-CH2 and one replicate for MUG-Chor1.

ChIP-seq data analysis. Genomic coordinates and gene annotation. All coordinates and gene annotations in this study were based on human reference genome assembly hg19, GRCh37 (ncbi.nlm.nih.gov/assembly/2758) and RefSeq genes.

ChIP-seq data processing. All datasets were aligned using Bowtie 2 (v.2.2.1) to build v.NCBI37/IG1911. Alignments were performed using all default parameters except -N 1.

Calculating read density. We calculated the normalized read density of a ChIP-seq dataset in any genomic region using the Bamulator (v.1.0) read density calculator (https://github.com/BradnerLab/pipeline/wiki/bamliquidator). Briefly, ChIP-seq reads aligning to the region were extended to 200 bp and the density
of reads per base pair was calculated. The density of reads in each region was normalized to the total number of million mapped reads producing read density in units of reads per million mapped reads per bp (rpm per bp).

### Plotting composite representations of ChIP-seq signal at individual loci.

To compactly display ChIP-seq signal from multiple cell line or patient-derived chordoma samples at individual genomic loci samples, we developed a simple meta representation as previously described. For all samples in a group, ChIP-seq signal is smoothed using a simple spline function and plotted as a translucent shape in units of rpm per bp. Darker regions indicate regions with signal in more samples. An opaque line is plotted and gives the average signal across all samples in a group.

### Identifying enriched regions.

We used the MACS v.1.4.2 (model-based analysis of ChIP-seq) peak finding algorithm to identify regions of ChIP-seq enrichment over background. A P value threshold of enrichment of 1e-9 was used for all datasets.

### Defining active genes.

Across chordoma cell lines, active genes were defined as those with an enriched H3K27ac region in at least one cell line in the ±1-kb region flanking the transcription start site.

### Mapping typical enhancers and super-enhancers using H3K27ac enhancer definitions in individual samples. H3K27ac super-enhancers and typical enhancers in individual chordoma samples were mapped using the ROSE2 software package described previously and available at https://github.com/BradnerLab/pipelineROSE2_main.py. For each dataset, a stitching parameter was determined that consolidated proximal peaks while optimizing the enriched fraction of stitched peaks. Peaks contained in the ±2.5-kb flanking the transcription start site of genes were excluded (~1,2500 parameter). To assign enhancers to the T or MAX genes, a 100-kb proximity window was used (Extended Data Figs. 4 and 6).

### Mapping typical enhancers and super-enhancers using H3K27ac enhancer definitions across compositions of cell lines or patient-derived chordoma samples.

H3K27ac super-enhancers and typical enhancers composite landscapes in either chordoma cell lines or patient-derived samples were mapped using the ROSE2 software package described previously and available at https://github.com/BradnerLab/pipelineROSE2_META.py. Briefly, for a given cohort of samples (for example cell lines), the union of all discrete H3K27ac enriched regions was determined. For each cohort of samples, a stitching parameter was determined that consolidated proximal peaks while optimizing the enriched fraction of stitched peaks. The average background subtracted signal for each sample across a cohort was averaged and used to rank enhancers. To assign enhancers to all genes, a 50-kb proximity window was used. Across chordoma cell lines, we identify a total of 1,199 super-enhancers (Fig. 3e and Supplementary Table 5). Across patient-derived chordoma samples, we identified 1,490 super-enhancers (Fig. 3g and Supplementary Table 7).

### Quantifying expression of super-enhancer-associated transcription factors.

Cell line expression data were provided by the Chordoma Foundation (available at https://www.cavatica.org). ChIP-seq reads were aligned to STAR-aligned reference genome (hg19) using Bowtie 2 (v.2.2.1). We aligned ChIP-seq reads to the human reference genome (hg19) using Bowtie 2 (v.2.2.1) and converted the aligned BAM files to BED files using BEDTools (v.2.26.0). Peaks were then called with MACS (v.1.4.2) annotated with nearby genes using HOMER (v.4.9.1) (http://homer.ucsd.edu/homer/). To be demarcated with a knockdown of (P < 0.05) in all three ChIP-seq replicates.

### Brachyury ChIP-seq analysis.

We obtained FASTQ files for a previously reported brachyury ChIP-seq experiment. We aligned ChIP-seq reads to the human reference genome (hg19) using Bowtie 2 (v.2.2.1) and converted the aligned BED files to BEDTools (v.2.26.0). Peaks were then called with MACS (v.1.4.2) annotated with nearby genes using HOMER (v.4.9.1) (http://homer.ucsd.edu/homer/). To be demarcated with a peak passing the significance threshold (P < 0.05) in all three ChIP-seq replicates.

### In vivo treatment studies.

All breeding, mouse husbandry and in vivo experiments were performed with the approval of the NYU Langone Medical Center Animal Care and Use Committee.

THZ1 was dissolved in DMSO:5% dextrose (1:10) and dosed as 40 mg kg$^{-1}$ twice daily via the intraperitoneal route. Vehicle-group mice received DMSO:5% dextrose (1:10) via the same route. The dosing schedule for the first week was 40 mg kg$^{-1}$ twice daily versus a non-targeting control must have had a log-fold change <0 and an adjusted P value <0.01. Brachyury ChIP-seq analysis was used as described in the associated Methods section.
Precellys Evolution (Bertin Technologies) instrument (3 × 7,500 r.p.m. for 20 s, pausing for 15 s between rounds). Homogenized lysates were centrifuged for 2 min at 15,700g. Supernatants were quantified and used for immunoblotting as described in the immunoblotting methods section. Immunoblots were performed once.

Studies were in compliance with all relevant ethical regulations.

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

**Code availability**

Computational code used for ChIP-seq analysis can be found at github.com/linlabcode/chordoma_code. Code for CRISPR-Cas9 screening analysis is available on GParc (www.gparc.org). Code for small-molecule sensitivity profiling analysis and DNA and RNA-sequencing analysis are available upon reasonable request.

**Data availability**

CRISPR-Cas9 screening data for two chordoma cell lines (pertains to Figs. 1, 2, 4 and Extended Data Fig. 1) were available at Figshare (https://doi.org/10.6084/m9.figshare.7302515). CRISPR-Cas9 screening data for all other cancer cell lines (pertains to Figs. 1, 2, 4 and Extended Data Fig. 1) were generated as part of Project Achilles (Broad Institute Project Achilles; https://depmap.org/portal/achilles/). All RNA-sequencing data (pertains to Figs. 1 and 3) are available at Gene Expression Omnibus (GEO) (accession number: GSE121846). Small-molecule sensitivity data generated using non-chordoma cell lines and used for comparative analyses (pertains to Fig. 2) are available at the National Cancer Institute's CTD2 Data Portal (https://ocg.cancer.gov/programs/ctd2/data-portal) and the CTRP (www.broadinstitute.org/ctrp/). The analysis of new small-molecule primary screening data generated using chordoma cell lines (pertains to Fig. 2) was performed as described previously26, except as noted in the Methods, and the resulting AUC values are provided in Supplementary Table 2. Raw plate-reader data files and accompanying Pipeline Pilot and MATLAB scripts for small-molecule primary screening and low-throughput compound sensitivity analysis (pertains to Fig. 2 and Extended Data Figs. 2a, 3a, 6c and 10a) are available upon reasonable request. Chromatin profiling data (pertains to Figs. 3 and 4 and Extended Data Figs. 4 and 6) are available at GEO (accession number: GSE109794).

**References**

41. Barretina, J. et al. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature* **483**, 603–607 (2012).
42. Doench, J. G. et al. Rational design of highly active sgRNAs for CRISPR-Cas9-mediated gene inactivation. *Nat. Biotechnol.* **32**, 1262–1267 (2014).
43. Cowley, G. S. et al. Parallel genome-scale loss of function screens in 216 cancer cell lines for the identification of context-specific genetic dependencies. *Sci. Data* **1**, 140035 (2014).
44. Aguirre, A. J. et al. Genomic copy number dictates a ene-independent cell response to CRISPR/Cas9 targeting. *Cancer Discov.* **6**, 914–929 (2016).
45. Johnson, W. E., Li, C. & Rabinovic, A. Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostatistics* **8**, 118–127 (2007).
46. Shalem, O. et al. Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science* **343**, 84–87 (2014).
47. Dancik, V. et al. Connecting small molecules with similar assay performance profiles leads to new biological hypotheses. *J. Biomol. Screen.* **19**, 771–781 (2014).
48. Dobin, A. et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**, 15–21 (2013).
49. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome. Biol.* **15**, 550 (2014).
50. Subramanian, A. et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc. Natl Acad. Sci. USA* **102**, 15545–15550 (2005).
51. Mootha, V. K. et al. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat. Genet.* **34**, 267–273 (2003).
52. Van der Auwera, G. A. et al. From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. *Curr. Protoc. Bioinformatics* **43**, 10 11–10 33 (2013).
53. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**, 1754–1760 (2009).
54. McKenna, A. et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* **20**, 1297–1303 (2010).
55. Langmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. *Nat. Methods* **9**, 357–359 (2012).
56. Zhang, Y. et al. Model-based analysis of ChIP-Seq (MACS). *Genome. Biol.* **9**, R137 (2008).
57. Brown, J. D. et al. NF-xB directs dynamic super enhancer formation in inflammation and atherogenesis. *Mol. Cell** **56**, 219–231 (2014).
58. Johannessen, C. M. et al. COT drives resistance to RAF inhibition through MAP kinase pathway reactivation. *Nature* **468**, 968–972 (2010).
59. Quinlan, A. R. & Hall, I. M. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* **26**, 841–842 (2010).
60. Heinz, S. et al. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol. Cell** **38**, 576–589 (2010).
Extended Data Fig. 1 | Dependency scores for sgRNAs targeting commonly essential genes. Viability after sgRNA treatment (represented by sgRNA-dependency scores; see Methods) corresponding to each of the primary screening sgRNAs targeting either RPS11 or RPS19 across 127 cancer cell lines (CCLs), including 2 chordoma CCLs (blue circles). For a given sgRNA, the median sgRNA-dependency score across 127 cell lines is indicated (gray line). Lower values indicate greater sgRNA depletion and thus essentiality of the target gene (shaded region).
Extended Data Fig. 2 | Sensitivity of chordoma cells to EGFR and/or ERBB2 inhibitors and of non-chordoma cells to CDK9 inhibitors. a, Validation of primary screening hit compounds targeting EGFR and/or ERBB2. Four chordoma cell lines were treated with indicated concentrations of candidate antiproliferative compounds and assayed for cell viability after 6 d with CellTiter-Glo. Response data are represented by a fitted curve to the mean fractional viability at each concentration relative to vehicle-treated cells; error bars represent the s.e.m. (n = 4 biological samples measured in parallel). b, Dinaciclib and alvocidib have antiproliferative effects across a wide range of cancer cell lines. AUC values corresponding to cell lines in CTRP treated with either dinaciclib or alvocidib. Each point represents a cancer cell line in CTRP treated with the indicated compound. Boxplots depict the inner quartiles (boxes) and median value (horizontal line) with whiskers representing 1.5 x the interquartile range of 445 (dinaciclib-treated) or 440 (alvocidib-treated) cell lines. AUCs were computed as described in the Methods and at https://github.com/remontoire-pac/ctrp-reference/tree/master/auc.
Extended Data Fig. 3 | Chordoma cells are less sensitive to CDK4/6 and CDK8/19 inhibitors. a, Response of chordoma cells to compounds targeting CDK4/6 and CDK8/19 proteins. Four chordoma cell lines were treated with indicated concentrations of compounds and assayed for cell viability after 6 d with CellTiter-Glo. Response data are represented by a fitted curve to the mean fractional viability at each concentration relative to vehicle-treated cells; error bars represent the s.e.m. (*n* = 4 biological samples measured in parallel). b, Immunoblot analysis of UM-Chor1 cells treated with indicated concentrations of inhibitors or DMSO for 24 h. The experiment was performed twice for CCT251545 (one representative experiment displayed) and once for other compounds.
Extended Data Figure 4 | T is super-enhancer-associated across chordoma cell lines. a, Enhancers in five chordoma cell lines ranked by H3K27ac signal in each sample. Enhancers proximal (within 100 kb) to the T gene start site are annotated, as described in the figure. Super-enhancers (SEs) were determined by the inflection point of the plot. b, Table showing the ranks of top T-associated enhancers in each chordoma sample.
Extended Data Fig. 5 | Brachyury is highly expressed in chordoma cell lines. 

**a**, Immunoblot analysis of chordoma and chondrosarcoma cell lines. Chordoma cell lines selectively express high levels of the brachyury protein. The experiment was performed once. 

**b**, Expression of T and MAX, as measured by RNA-sequencing, across 935 non-chordoma cancer cell lines derived from diverse tumor types. Data were generated as part of the Broad Institute Cancer Cell Line Encyclopedia (quantified data obtained from: https://ocg.cancer.gov/ctd2-data-project/translational-genomics-research-institute-quantified-cancer-cell-line-encyclopedia). Boxplots depict the inner quartiles (boxes) and median value (horizontal line) with whiskers representing 1.5× the interquartile range.

**c**, Gene-expression levels of 115 super-enhancer- (SE-) associated transcription factors in five chordoma cell lines (points), ranked by mean expression (horizontal ticks). 

**d**, T is amplified in the JHC7 chordoma cell line. Genomic copy-number alterations, inferred from whole-exome sequencing data, in five chordoma cell lines. A region of 2.06 Mb around the T locus on chromosome 6 shows 26-fold amplification in JHC7. This finding is consistent with the 2.6-Mb amplicon inferred from ChIP-seq whole-cell extract.
Extended Data Fig. 6 | T is super-enhancer-associated in patient-derived chordoma tumors. a, Enhancers in chordoma tumors ranked by H3K27ac signal in each sample. Super-enhancers (SEs, red) and typical enhancers (black) proximal (within 100 kb) to the T gene start site are annotated. Super-enhancers were determined by the inflection point of the plot. b, Table showing the ranks of top T-associated super-enhancers or typical enhancers in each chordoma sample.
Extended Data Figure 7 | Patient-derived chordoma tumors express brachyury. Immunohistochemical staining of patient-derived chordoma tumors for brachyury expression. H&E, hematoxylin and eosin. The experiment was performed once.
Extended Data Fig. 8 | THZ1 and actinomycin D reduce expression of T (brachyury) in a concentration- and time-dependent fashion. a, Immunoblot analysis of UM-Chor1 cells treated with indicated concentrations of THZ1 or DMSO for 12, 24, 36 or 48 h. The experiment was performed once. b, UM-Chor1 cells were treated with indicated concentrations of compound or DMSO for 4, 8 or 12 h and subjected to RT-qPCR. Data are expressed as the log₂ fold change of transcript levels relative to vehicle-treated cells, normalized to GAPDH levels, and represent the mean ± s.d. (n = 3 biological samples measured in parallel). Results of statistical analyses of RT-qPCR data, derived from a one-sided Welch’s t-test, are reported in Supplementary Table 9. c, Response of chordoma cells to treatment with actinomycin D. Four chordoma cell lines were treated with indicated concentrations of compound and assayed for cell viability after 6 d with CellTiter-Glo. Response data are represented by a fitted curve to the mean fractional viability at each concentration relative to vehicle-treated cells; error bars represent the s.e.m. (n = 4 biological samples measured in parallel).
Extended Data Fig. 9 | Expression of ATP6V1B2, SAE1, SOX9 and TPX2 is downregulated following sgRNA-mediated T (brachyury) repression. a, UM-Chor1 cells were transduced with sgRNAs targeting T or a non-targeting sgRNA control and subjected to RT–qPCR. Data are expressed as the fold-change of transcript levels relative to sgRNA control-treated cells, normalized to GAPDH levels and represent the mean (n=2 biological samples measured in parallel, represented by black points). * P<0.05; ** P<0.01; *** P<0.001; P values were derived from a one-sided Welch’s t-test. Exact P values and effect sizes are reported in Supplementary Table 9. b, Immunoblot analysis of UM-Chor1 cells transduced with sgRNAs targeting T or a non-targeting sgRNA control. SgRNA treatment was performed once and immunoblotting was performed twice (one representative experiment displayed).
Extended Data Fig. 10 | THZ1 treatment can reduce brachyury expression in CH22 cells ex vivo and in vivo. a, CH22 chordoma cells were treated with indicated concentrations of transcriptional CDK inhibitors and assayed for cell viability after 6 d with CellTiter-Glo. Response data are represented by a fitted curve to the mean fractional viability at each concentration relative to vehicle-treated cells; error bars represent the s.e.m. (n = 4 biological samples measured in parallel). b, Immunoblot analysis of CH22 cells treated with indicated concentrations of inhibitors targeting CDK4/6 (palbociclib), CDK7/12/13 (THZ1) or CDK9 (dinaciclib, NVP-2, alvocidib) or DMSO for 48 h. The experiment was performed once. c, Weight change of mice treated with THZ1 or vehicle for the study depicted in Fig. 4h,i. d, THZ1 can downregulate brachyury expression in vivo. Immunoblot analysis of CH22 xenograft tumors following treatment with indicated doses of THZ1 or vehicle twice daily for 5 d. The experiment was performed once. e, Immunoblot analysis of CH22 xenograft tumors following treatment with THZ1 or vehicle twice daily for 3 d. Top and bottom panels represent two independent studies (bottom panel corresponds to the study depicted in Fig. 4h,i).
Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

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- An indication of 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
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- A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. $F$, $t$, $r$) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted
  - *Give $P$ values as exact values whenever suitable.*
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s $d$, Pearson’s $r$), indicating how they were calculated
- Clearly defined error bars
  - *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

| Data collection |
|-----------------|
| Luminescence measurements were collected using EnVision Workstation Software (PerkinElmer); RT-qPCR measurements were collected with QuantStudio 6 and 7 Flex Software (Applied Biosystems); immunoblot scans were performed with Image Studio Software (LI-COR Biosciences); low-throughput cell counting (pertains to Fig. 1c) was performed with Vi-CELL XR Software (Beckman Coulter); high-resolution digital immunohistochemical staining images were generated using Leica ScanScope XT. |
Data

Policy information about availability of data

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

CRISPR-Cas9 screening data for two chordoma cell lines (pertains to Figs. 1, 2, 4, and Extended Data Fig. 1) are available at Figshare (DOI: 10.6084/m9.figshare.7302515). CRISPR-Cas9 screening data for all other cancer cell lines (pertains to Figs. 1, 2, 4, and Extended Data Fig. 1) were generated as part of Project Achilles (Broad Institute Project Achilles; https://depmap.org/portal/achilles/). All RNA-sequencing data (pertains to Figs. 1 and 4) are available at Gene Expression Omnibus (GEO) (accession number: GSE121846). Small-molecule sensitivity data generated using non-chordoma cell lines and used for comparative analyses (pertains to Fig. 2) are available at the National Cancer Institute’s CTD2 Data Portal (https://ocg.cancer.gov/programs/ctd2/data-portal) and the CTRP (www.broadinstitute.org/ctrp/). The analysis of new small-molecule primary screening data generated using chordoma cell lines (pertains to Fig. 2) was performed with Bowtie2 (version 2.2.1); Bamliquidator (version 1.0); MACS (version 1.4.2); ROSE2 (https://github.com/BradnerLab/pipeline); STAR; and Sailfish. ATAC-seq data analysis was performed with Bowtie2 (version 2.2.1); samtools (0.1.19); and MACS (version 1.4.2). Brachyury ChIP-seq analysis was performed with Bowtie 2 (version 2.2.1); Bedtools (version 2.26.0); MACS (version 1.4.2); and HOMER (version 4.3.1) (http://homer.ucsd.edu/homer/). All other statistical analyses were performed with GraphPad Prism (v6 or v7); or R (version 3.4.4).

Field-specific reporting

Please select 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

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Life sciences study design

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

Sample size

Sample sizes were determined on the basis of previous studies that had used similar sample sizes to successfully detect a difference between groups.

Data exclusions

For primary small-molecule screening, individual microtiter wells not meeting routine quality control standards of our high-throughput screening facility were excluded from analysis. For in vivo studies, one mouse in the vehicle group did not have successful tumor engraftment and was therefore excluded from the study.

Replication

Measures taken to verify the reproducibility of the findings included some combination of the following: confirming an effect across multiple cell lines or tumor samples, replicating findings with independent experiments, using biological and technical replicates, using multiple concentrations of a compound, using multiple sgRNAs targeting a gene of interest, and confirming ex vivo results in different model systems (patient-derived tumors or mice). All attempts at replication were successful.

Randomization

Human research participants were not grouped. Animals were randomly grouped.

Blinding

Human research participants were not grouped. Blinding was not possible for animal studies, as animals were segregated into distinct, labeled cages according to the treatment group.

Reporting for specific materials, systems and methods
**Materials & experimental systems**

| n/a | Involved in the study |
|-----|------------------------|
| ✗   | Unique biological materials |
| ✗   | Antibodies |
| ✗   | Eukaryotic cell lines |
| ✗   | Palaeontology |
| ✗   | Animals and other organisms |
| ✗   | Human research participants |

**Methods**

| n/a | Involved in the study |
|-----|------------------------|
| ✗   | ChIP-seq |
| ✗   | Flow cytometry |
| ✗   | MRI-based neuroimaging |

**Antibodies**

Antibodies used: For immunoblotting: the goat polyclonal brachyury antibody (clone N-19, #sc-17743; 1:1,000) and the mouse monoclonal CDK7 antibody (clone C-4, #sc-7344; 1:1,000) were purchased from Santa Cruz Biotechnology. The rabbit monoclonal coflin antibody (clone D3F9, #5175; 1:10,000), the mouse monoclonal total Rb antibody (clone 4H1, #9309; 1:2,000), the rabbit monoclonal phospho-Rb S807/S811 antibody (clone D20B12, #8516; 1:1,000), the rabbit monoclonal phospho-Rb S780 antibody (clone D5987, #8180; 1:1,000), the mouse monoclonal total STAT1 antibody (clone 9H2, #9176; 1:1,000), the rabbit monoclonal phospho-STAT1 S727 antibody (clone D3B7, #8826; 1:1,000), and the rabbit monoclonal SOX9 (clone D8G8H, #82630; 1:1,000) were purchased from Cell Signaling Technology. The rat monoclonal phospho-POLR2A Ser2 antibody (clone 3E1, #04-1571-I; 1:1,000), the rat monoclonal phospho-POLR2A Ser5 antibody (clone 3E8, #04-1572-I; 1:1,000), and the rat monoclonal phospho-POLR2A Ser7 antibody (clone 4E12, #04-1570-I; 1:1,000) were purchased from Millipore. The rabbit polyclonal total POLR2A antibody (#A300-653A; 1:400) was purchased from Bethyl Laboratories. The mouse monoclonal V5-tag antibody (#46-0705; 1:5,000) was purchased from Invitrogen. IRDye secondary antibodies were purchased from LI-COR Biosciences (1:10,000). For ChIP-seq: the rabbit polyclonal H3K27Ac antibody (#ab4729) was purchased from abcam. For immunohistochemistry, the goat polyclonal brachyury antibody (clone N-19, #sc-17743) was purchased from Santa Cruz Biotechnology. Lot numbers were not routinely recorded.

**Validation**

All antibodies are commercially available and have been validated by their manufacturers as described on the company websites. In addition, the brachyury antibody (clone N-19, #sc-17743) was validated using sgRNA-mediated T (brachyury) repression experiments as reported in Fig. 1c.

**Eukaryotic cell lines**

Policy information about [cell lines](#).

**Cell line source(s)**

UM-Chor1, MUG-Chor1, U-CH1, U-CH2, and JHC7 chordoma cell lines were provided by the Chordoma Foundation; the CH22 chordoma cell line was provided by the Chordoma Foundation and Massachusetts General Hospital; chondrosarcoma cell lines were obtained from the Broad Institute Cancer Cell Line Encyclopedia project (Barretina et al.; DOI: 10.1038/nature11003).

**Authentication**

All chordoma cell lines were authenticated by the Chordoma Foundation using the following criteria: each line must have a confirmed diagnosis of chordoma, be of human origin, and have a rearranged genome. Additionally, the line must be immortal, have gone through at least 50 population doublings in culture, and have a genotype that does not match any known cell line by STR analysis. Furthermore, at least two of the following criteria must be met: expression of brachyury, expression of CD24, evidence of a physaliferous morphology, or evidence of genomic rearrangement that matches the original tumor. Chondrosarcoma cell lines were authenticated by the Cancer Cell Line Encyclopedia project as described by Barretina et al. (DOI: 10.1038/nature11003).

**Mycoplasma contamination**

Chordoma cell lines were tested for mycoplasma contamination by the Chordoma Foundation and were determined to be mycoplasma-negative. Chondrosarcoma cell lines were tested for mycoplasma contamination by the Cancer Cell Line Encyclopedia project and were determined to be mycoplasma-negative, as described by Barretina et al. (DOI: 10.1038/nature11003).

**Commonly misidentified lines**

(See ICLAC register)

No commonly misidentified cell lines were used.

**Animals and other organisms**

Policy information about [studies involving animals; ARRIVE guidelines](#) recommended for reporting animal research.

**Laboratory animals**

SCID Hairless Outbred (SHO), all female, 6-8 week-old mice were purchased from Charles River Laboratories International Inc. (#474).

**Wild animals**

The study did not involve wild animals.

**Field-collected samples**

The study did not involve samples collected from the field.
Human research participants

Policy information about studies involving human research participants

Population characteristics

Relevant information on human research participants is provided in Supplementary Table 6. Covariate-relevant population characteristics of the participants provided include: age, sex, tumor location, classification as metastatic or non-metastatic disease, classification as recurrent or non-recurrent disease, and preoperative treatment.

Recruitment

Patient-derived tissue was obtained from Massachusetts General Hospital (MGH) and the Chordoma Foundation (CF) as described in the Methods. Tissue from MGH was obtained based on availability; tissue from CF was requested to be from a mixture of anatomical locations, non-metastatic, non-recurrent, and untreated.

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.

Files in database submission

.wig and .fastq files are provided.

Genome browser session

(e.g. UCSC)

No longer applicable.

Methodology

Replicates

ChIP-seq analysis was performed on 5 cell lines, 8 patient-derived tumor samples, and 1 patient-derived normal adjacent tissue sample (1 replicate each). ATAC-seq analysis was performed on two cell lines (2 biological replicates for U-CH2 and 1 replicate for MUG-Chor1).

Sequencing depth

All ChIP-seq datasets were sequenced with 75 bp single-end reads. All ATAC-Seq datasets were sequenced with 100 bp paired-end reads. The associated table provides read depths for each dataset in units of millions of reads.

Antibodies

ChIP-seq was performed using an anti-H3K27ac antibody (abcam, #ab4729).

Peak calling parameters

MACS 1.4.2 was applied with a p-value cutoff of 1e-9.

Data quality

Only high confidence peaks (p-value cutoff of 1e-9) were used in subsequent analysis. To ensure quality, super-enhancer analysis was aggregated across all cell lines or all patient-derived samples (see Methods). The rank of the brachyury-associated super-enhancer is also shown across multiple individual samples.

Software

ChIP-seq analysis was performed with Bowtie2 (version 2.2.1); Bamliquidator (version 1.0); MACS (version 1.4.2); ROSE2 (https://github.com/BradnerLab/pipeline); STAR; and Sailfish. ATAC-seq data analysis was performed with Bowtie2 (version 2.2.1); samtools (0.1.19); and MACS (version 1.4.2). All relevant processing scripts can be found at https://github.com/linlabcode/chordoma_code.