LETTERS

CDK8 is a colorectal cancer oncogene that regulates β-catenin activity

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Aberrant activation of the canonical WNT/β-catenin pathway occurs in almost all colorectal cancers and contributes to their growth, invasion and survival[1,2]. Although dysregulated β-catenin activity drives colon tumorigenesis, further genetic perturbations are required to elaborate full malignant transformation[3]. To identify genes that both modulate β-catenin activity and are essential for colon cancer cell proliferation, we conducted two loss-of-function screens in human colon cancer cells and compared genes identified in these screens with an analysis of copy number alterations in colon cancer specimens. One of these genes, *CDK8*, which encodes a member of the mediator complex[4], is located at 13q12.13, a region of recurrent copy number gain in a substantial fraction of colon cancers. Here we show that the suppression of *CDK8* expression inhibits proliferation in colon cancer cells characterized by high levels of CDK8 and β-catenin hyperactivity. CDK8 kinase activity was necessary for β-catenin-driven transformation and for expression of several β-catenin transcriptional targets. Together these observations suggest that therapeutic interventions targeting CDK8 may confer a clinical benefit in β-catenin-driven malignancies.

To identify oncogenes that modulate β-catenin-dependent transcription and regulate colon cancer cell proliferation, we conducted two RNA interference (RNAi)-based loss-of-function screens. We engineered DLD-1 colon cancer cells, which harbour *APC* deletions

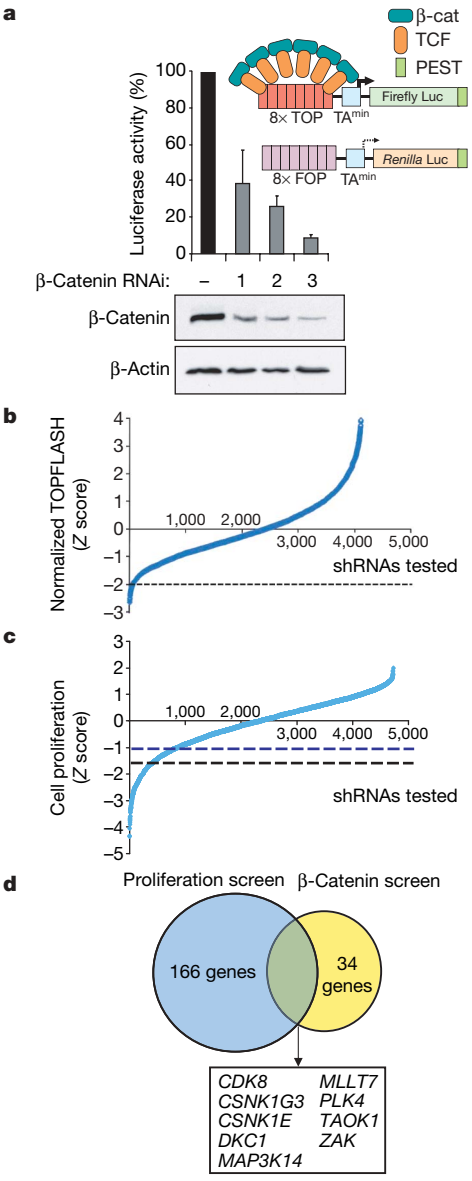

Figure 1 | RNAi screens to identify genes essential for colon cancer cell proliferation and β-catenin activity. **a**, Schematic of the DLD-1^{Rep} cell line showing the engineered 8× TOPFLASH (TOP) and 8× FOPFLASH (FOP) elements and relative TOP/FOP activity in the DLD-1^{Rep} cell line. TA, minimal TA promoter; PEST, domain rich in proline (P), glutamate (E), serine (S) and threonine (T) residues. **b**, Distribution curve showing *Z* scores representing β-catenin activity for all shRNAs tested in the DLD-1^{Rep} screen. shRNAs that reduced FOPFLASH levels to near background, or activated FOPFLASH more than 2 s.d. above the mean, were excluded (FOP ≤800 and FOP ≥2,600 luciferase units). Two out of five *CDK8*-specific shRNAs were excluded on this basis. shRNA that induced *Z* scores of >4 are not shown. The dashed line indicates the *Z* score cutoff for shRNAs scored as hits. **c**, Distribution curve showing *Z* scores representing cell proliferation for shRNAs tested in HCT116 cells. This screen contained shRNAs targeting 1,004 genes, and there was a 92% overlap between the screens in **b** and **c**. The blue and black dashed lines indicate a *Z* score cutoff for shRNAs scored as hits. **d**, Venn diagram representation of the nine genes that reduced both β-catenin activity and colon cancer cell proliferation.

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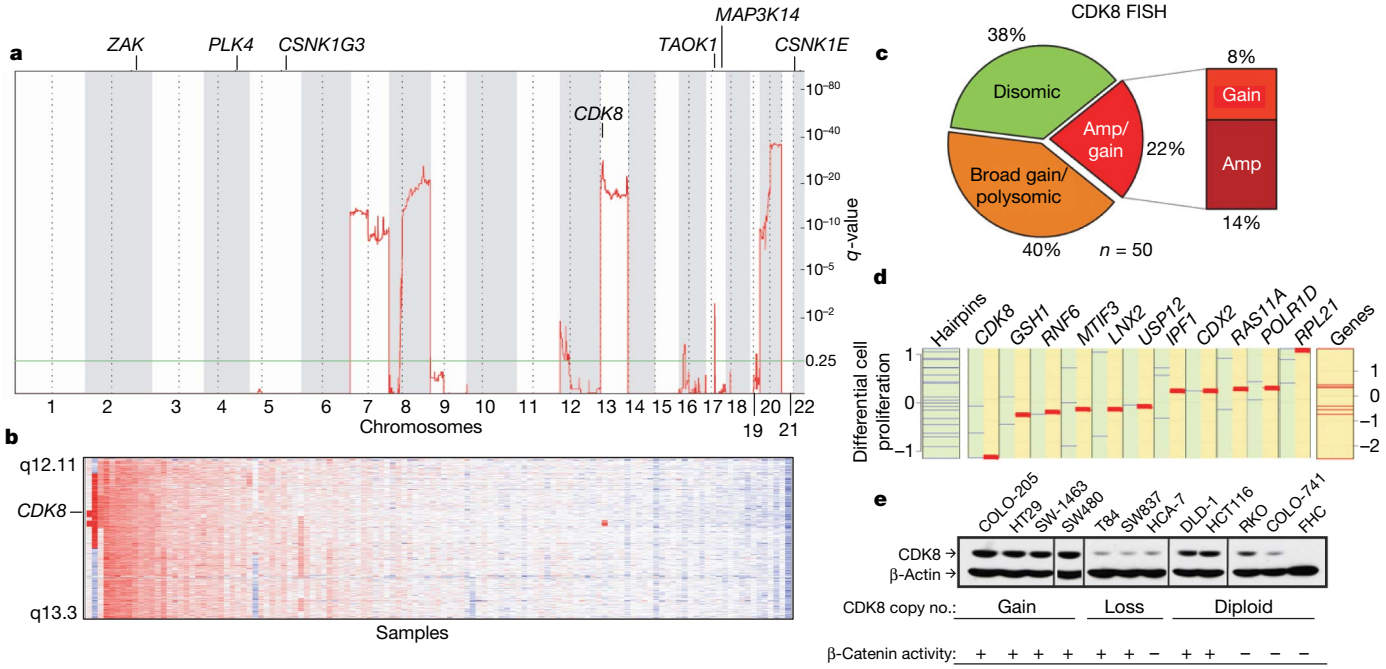
and depend on β-catenin for proliferation[6], to stably express 'pTOPFLASH' β-catenin–luciferase and 'pFOPFLASH' mutant-*Renilla* reporter constructs[7,8] (DLD-1^{Rep}). Suppression of β-catenin expression in DLD-1^{Rep} cells by three β-catenin-specific short hairpin RNAs (shRNA) markedly reduced the TOPFLASH/FOPFLASH ratio (Fig. 1a), confirming that reporter activity requires β-catenin expression. We then screened DLD-1^{Rep} cells with a shRNA library containing 4,849 shRNAs that target 1,000 genes, including 95% of all human kinases[7]. We found 34 genes which had expression that was necessary for β-catenin activity, including two known β-catenin regulators, *CSNK1G3* (ref. 9) and *CSNK1E* (ref. 10; Fig. 1b and Supplementary Table 1).

In parallel, we performed an arrayed, kinase-enriched shRNA screen in another β-catenin-dependent colon cancer cell line, HCT116, to identify genes essential for cancer cell proliferation. We identified 166 candidate genes necessary for proliferation using the criteria that at least two shRNAs targeting the same gene induced a decrease in proliferation. Among the genes identified in this screen were the oncogenes *KRAS* and *MYC* (Fig. 1c and Supplementary Table 2). Combining the genes from the two screens showed that there were nine genes for which suppression affected both β-catenin transcriptional activity and colon cancer cell proliferation (Fig. 1d).

To determine whether any of these genes are amplified in colon cancers, we used single nucleotide polymorphism (SNP) arrays and the GISTIC[11,12] statistical method to conduct a genome-wide analysis of autosomal copy number alterations in primary resection specimens from 123 human colorectal adenocarcinomas (Fig. 2a). Among the nine genes identified by our RNAi screens, only *CDK8* resides in a significant amplicon at 13q12.13–13q12.2 (false discovery rate $= 1 \times 10^{-29}$; Fig. 2a). These findings confirm recent reports that a large portion of chromosome 13 is amplified in colon cancers[13,14]. A total of 58 out of 123 (47%) samples harboured this region of copy number gain (Fig. 2b and Supplementary Table 3).

To confirm these findings, we performed fluorescence *in situ* hybridization (FISH), using probes specific for *CDK8* and *RB1* (chromosome 13 control probe), on a tissue microarray (TMA) carrying



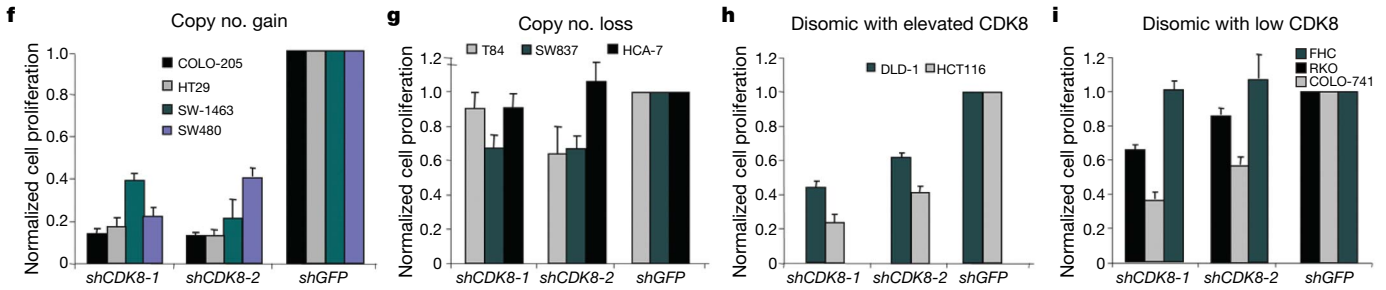


Figure 2 | Amplification and overexpression of CDK8 defines a subset of colon cancers. a, The significance (*y* axis; *q*-value) of recurrent amplifications at loci across the 22 autosomes (*x* axis) identified by GISTIC analysis. The chromosomal location of RNAi hits is indicated above the plot. **b**, Heat map showing clustering of SNP array data, on the basis of chromosome 13q12 copy number in 123 colon cancer specimens. Red indicates allelic gain, blue denotes allelic loss. **c**, Pie chart depicting the percentage of colon tumours with *CDK8* copy number gain, chromosome 13 polysomy or disomy ($n = 50$) by FISH. Cutoff criteria for FISH are shown in Supplementary Table 4. Amp, genomic amplification. **d**, GSEA comparative analysis of suppressing resident genes in the minimal 13q12 region. Blue lines represent differential scores of cell proliferation effects for each validated shRNA that suppressed target gene expression by more than 70%. The left-most column demonstrates the total pool of validated hairpins for all genes on 13q12. The red lines represent a normalized enrichment score for each gene that takes into account the cell proliferation effects of all shRNAs and scores the specificity of the effects in cell lines that harbour or lack 13q gain. **e**, Immunoblot analysis of CDK8 expression in 12 colon cancer cell lines; β-actin was used as a loading control. **f**–**i**, Effect of *CDK8* suppression on proliferation of colon cancer cells that harbour chromosome 13 copy number gain (**f**), loss (**g**), are disomic with increased CDK8 protein expression (**h**) and are disomic with reduced CDK8 protein expression (**i**). Bar graph depicts cell proliferation normalized to the *shGFP* control for triplicate determinations. Error bars represent mean ± s.d. for a representative experiment performed in triplicate.

50 evaluable colon cancer specimens. We detected *CDK8* copy number gain in 31 out of 50 (62%) cases. Of these 50 cases, 20 showed gains in both *CDK8* and *RB1*, indicative of polysomy and consistent with recent observations linking RB, E2F1, β-catenin and CDK8 (ref. 15). We also found *CDK8* amplifications in seven of these tumours (defined as a CDK8:control ratio ≥2) and low to moderate level copy number gain (a CDK8:control ratio >1 and <2) in a further four specimens (Fig. 2c and Supplementary Table 4). Immunohistochemical analysis of CDK8 expression in the same 50 specimens showed elevated protein levels in 13 out of 50 (26%) colon cancer samples, including those that showed *CDK8* copy number gain (Supplementary Fig. 1 and Supplementary Table 4). These observations indicate that *CDK8* is amplified and overexpressed in a substantial fraction of colon cancers.

The minimal region shared by these tumours encompasses 16 annotated genes (Supplementary Fig. 2a). To determine whether *CDK8* is the primary target of this amplicon, we first assessed expression of these genes in colon cancer cells harbouring chromosome 13q copy number gain and found that four of the genes were not expressed (Supplementary Fig. 2b). We suppressed the expression of the remaining 12 genes in four cell lines: two (HT29 and COLO-205) that harbour 13q copy number gain, and two (SW837 and T84) that show 13q loss (Supplementary Fig. 3a, b). To analyse the screen results on a per-gene basis in cell lines with either 13q12 copy number gain or deletion, we used an adaptation of the GSEA[16] method and found that *CDK8* was the only gene in this region that was required for proliferation of cell lines harbouring 13q gain (false discovery rate = 0.24; Fig. 2d and Supplementary Table 5). These observations suggested that colon cancer cells harbouring 13q12.2 amplification are particularly dependent on CDK8 expression for proliferation.

We then analysed *CDK8* copy number gain and protein expression in a panel of 12 colon cancer lines. Four (COLO-205, HT29, SW1463 and SW480)[17] of these twelve lines were found to harbour *CDK8* gain (Supplementary Fig. 3a, b), and these cell lines showed the highest amounts of CDK8 protein (Fig. 2e). Two additional colon cancer cell lines disomic for *CDK8* (DLD-1 and HCT116) also had elevated CDK8 protein amounts (Fig. 2d and Supplementary Fig. 3a, b). Suppression of *CDK8* expression induced substantially decreased proliferation in all six cell lines with elevated CDK8 protein concentrations (Fig. 2f, g) but inhibited proliferation rates in the cell lines with lower CDK8 protein levels to a lesser degree (Fig. 2h, i). Suppressing CDK8 in colon cancer cells reduced the fraction of cells in G1 and S phase, increased the number of aneuploid cells and markedly slowed cell proliferation without inducing apoptosis (Supplementary Fig. 4), similar to what was observed on suppression of β-catenin. These observations demonstrate that colon cancer cells that express elevated CDK8 levels are highly dependent on its expression for proliferation.

To determine whether CDK8 induces cell transformation, we overexpressed wild-type CDK8 or a previously reported kinase-inactive substitution mutant (D173A; CDK8-KD)[18] in immortal murine fibroblasts (NIH 3T3 cells; Fig. 3a). CDK8 expression induced focus formation, anchorage-independent colony growth and tumour formation in immunodeficient animals (Fig. 3b–e), whereas the CDK8-KD mutant failed to transform the cells. These observations confirm that CDK8 is a bona fide oncogene, the kinase activity of which is necessary for oncogenic activity.

To dissect the relationship between CDK8 and β-catenin activity, we measured endogenous β-catenin activity in the 12 cell lines used above. The RKO, COLO-741, HCA-7 and FHC cell lines do not harbour known APC or β-catenin mutations[19–21] and, as predicted, had low amounts of β-catenin activity. Of these four cell lines, suppression of *CDK8* induced a substantial proliferation effect only in COLO-741 (Fig. 2h and Supplementary Fig. 5a). Similarly, of the 12 cell lines tested, the six cell lines with the highest CDK8 elevation showed a greater dependence on β-catenin for proliferation (Supplementary Fig. 5b).

CDK8 is a cyclin-dependent kinase member of the mediator complex, which couples transcriptional regulators to the basal transcriptional machinery[4]. To explore the role of CDK8 in modulating β-catenin transcriptional activity, we confirmed that suppressing CDK8 with two independent, *CDK8*-specific shRNAs (*shCDK8-1* and *shCDK8-2*) in another cell line, SW480, also reduced β-catenin-dependent transcriptional activity (Fig. 4a). CDK8 kinase activity depends on the co-factor cyclin C[22], and we found that cyclin C knockdown preferentially affected colon cancer cell lines with chromosome 13q gain (Supplementary Fig. 6a, b). To test whether CDK8 kinase activity is required to regulate β-catenin activity, we expressed wild-type CDK8 or CDK8-KD in DLD-1^{Rep} cells carrying a shRNA targeting the 3′-untranslated region, *shCDK8-1*, and found that only wild-type CDK8 partially rescued the effects of suppressing endogenous *CDK8* (Supplementary Fig. 6c). These observations demonstrate that the kinase activity of CDK8 is necessary for both CDK8-induced transformation and β-catenin driven transcription.

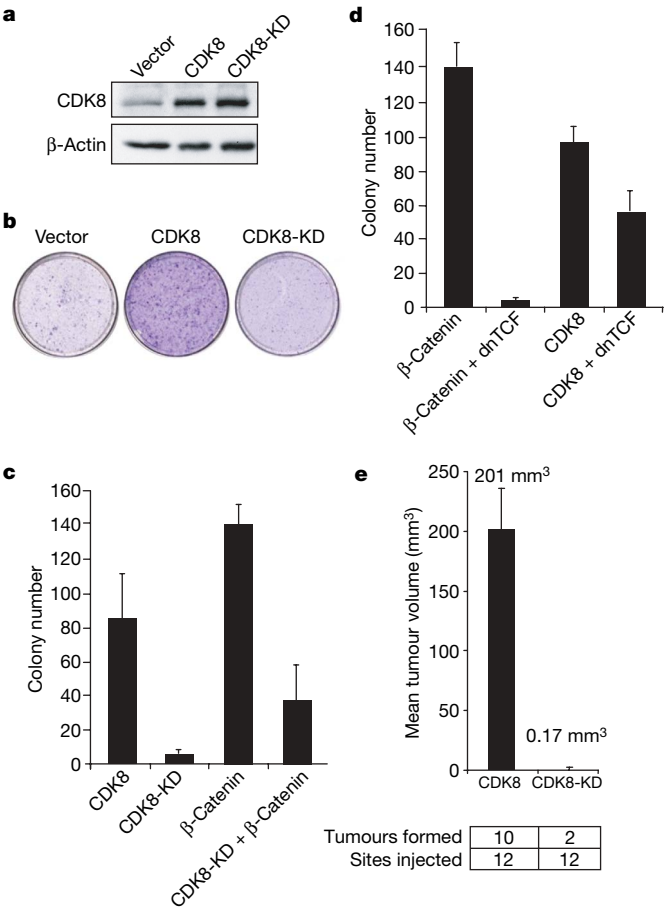


Figure 3 | CDK8 and transformation. **a**, Immunoblot analysis showing CDK8 and CDK8 kinase-dead (CDK8-KD) expression in NIH 3T3 cells. **b**, Focus formation assay of NIH 3T3 cells expressing CDK8 or CDK8-KD. **c**, CDK8 kinase activity drives anchorage-independent growth and is necessary for β-catenin (CTNNB1) mediated anchorage-independent growth. Anchorage-independent colony growth in NIH 3T3 cells infected with the indicated retroviral vectors is shown. **d**, β-Catenin suppression only partially blocks CDK8-induced anchorage-independent growth. Dominant negative TCF (dnTCF) was introduced in the presence of β-catenin or CDK8. Anchorage-independent colony growth was performed as indicated in **c**. **e**, CDK8 kinase activity drives tumour formation. Mean tumour volume from subcutaneous tumours formed NIH 3T3 cells expressing CDK8 or CDK8-KD constructs in immunodeficient mice. The difference in tumour formation between CDK8 and CDK8-KD was statistically significant, as assessed by an unpaired *t*-test (*P* value = 0.0001). All experiments were performed in triplicate and mean ± s.d. are shown.

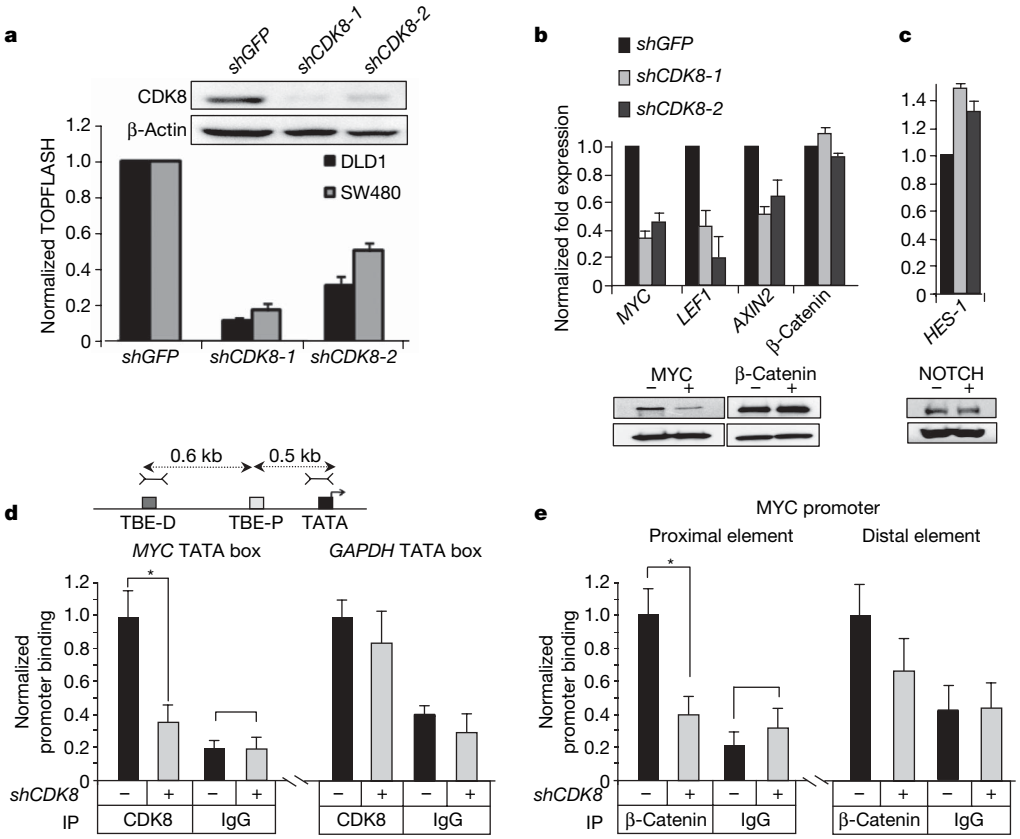


Figure 4 | CDK8 mediates transcription of β-catenin-driven downstream target genes. a, Bar graph depicts β-catenin activity as normalized TOPFLASH (TOPFLASH/FOPFLASH) after introduction of *CDK8*-specific shRNAs. An immunoblot shows CDK8 protein amounts at the time of the assay; β-actin was used as a loading control. **b**, **c**, Bar graphs show messenger RNA abundance of endogenous β-catenin and NOTCH transcriptional targets after CDK8 suppression in COLO-205 and DLD-1 cells. MYC, β-catenin and NOTCH protein expression changes were assessed by immunoblotting; β-actin expression was used as a loading control. **d**, CDK8 binds the *MYC* promoter. Schematic representation depicts the location of proximal and distal TCF binding elements (TBE, grey boxes) and *MYC* promoter TATA box. Dashed lines indicate the distance from TBEs to the TATA box. Hatched lines depict the PCR primers used for ChIP. The bar graph shows CDK8 binding to the *MYC* TATA box in COLO-205 colon cancer cells treated with the indicated shRNAs and assayed by ChIP. **e**, Bar graph depicts β-catenin binding to the indicated *MYC* promoter elements in the presence or absence of CDK8. Asterisks indicate statistical significance as assessed by an unpaired Student's *t*-test ($P \leq 0.013$ for CDK8 and $P \leq 0.14$ for the IgG control in **d**; $P \leq 0.005$ for CDK8 and $P \leq 0.34$ for the IgG control in **e**). ChIP assays were performed as described earlier. All experiments were conducted at least twice in triplicate. Error bars represent the mean ± s.d. for a representative experiment performed in triplicate. IP, immunoprecipitate.

The TCF–β-catenin complex regulates expression of several genes implicated in colon cancer, including *MYC*[23], *AXIN2* (ref. 24) and *LEF1* (ref. 25). Suppression of CDK8 in DLD-1 and COLO-205 cells reduced expression of each of these genes (Fig. 4b). In contrast, we failed to observe changes in the expression of NOTCH or HES-1 (Fig. 4c), previously reported targets of CDK8 (ref. 26). Thus, CDK8 modulates a subset of β-catenin-driven genes previously implicated in cancer[23,24,27].

We then performed chromatin immunoprecipitation (ChIP) near two verified β-catenin/TCF binding elements (TBE)[25] in the *MYC* promoter, as an example of a β-catenin regulated gene, to test whether CDK8 directly modulates *MYC* expression at the promoter level. We found that CDK8 associated with the *MYC* promoter (Fig. 4d). We therefore asked if loss of CDK8 binding at the *MYC* promoter affects the ability of β-catenin to bind at the proximal and distal TBEs. Suppression of *CDK8* expression reduced the amount of β-catenin bound to the proximal element in the *MYC* promoter but had little effect on the amount associated with the distal element (Fig. 4e). These observations implicate CDK8 and the mediator complex[4] as a direct regulator of β-catenin-driven transcription.

To test whether CDK8 activity is also required for β-catenin-driven transformation, we expressed the dominantly interfering CDK8-KD mutant[28] in transformed NIH 3T3 cells expressing a constitutively active β-catenin mutant (Fig. 3c). Disruption of CDK8 activity inhibited β-catenin-driven transformation, whereas a dominantly interfering TCF construct, previously shown to inhibit β-catenin-induced cellular transformation[29], only partially abrogated CDK8-mediated transformation (Fig. 3d). These observations suggest that although CDK8 is required for β-catenin-mediated transformation, the full capacity of CDK8 to transform cells may extend beyond its ability to activate β-catenin.

We have used an integrated approach to identify *CDK8* as an oncogene in a substantial fraction of colorectal cancers and demonstrate that the kinase activity of CDK8 is essential for it to be able to regulate β-catenin-dependent transcription and transformation. These observations indicate that CDK8 acts in part by co-activating β-catenin-driven transcription in colon cancers characterized by both high CDK8 expression and β-catenin activity. Accordingly, therapeutic interventions that target the CDK8 kinase activity in such cancers may be of clinical value.

METHODS SUMMARY

Lentiviral infections were performed using pLKO.1 lentiviral shRNA constructs generated by the RNAi consortium[7] and are listed in Supplementary Table 6. For high-throughput screening, cells infected with lentiviruses were allowed to grow for either 4 days (DLD-1^{Rep}) or 5 days (HCT116). Suppression of β-catenin activity was defined as the ability of at least one shRNA to decrease activity more than 2 s.d. below the *Z* score. Suppression of proliferation was defined as the capacity for at least one shRNA to decrease proliferation more than 1.5 s.d. below the mean *Z* score and at least one extra shRNA targeting the same kinase to decrease proliferation more that 1 s.d. below the mean *Z* score. For tissue

analyses, a TMA composed of human colon cancer tissue and matching patient normal colon was subjected to immunohistochemical staining. For FISH, BAC clones were hybridized in dual colours to 4-μm TMA sections as follows: the RB1 probe labelled in SpectrumGreen was used as a chromosome 13 control probe and the RP11-726I20 BAC, spanning CDK8, was labelled with SpectrumOrange dUTP (both from Abbott Molecular/Vysis).

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions R.F., A.J.B., M.M. and W.C.H. designed the approach. R.F., A.J.B., E.B., E.F., I.F.D., I.G., J.S.B., N.V., S.J.S., S.O., S.Y.K. and Y.S. performed the experiments. J. Barretina, J. Baselga, J.T. and R.A.S. provided samples and analysis. A.H.L., A.J.B., C.M., C.S.F., D.E.R., E.B., E.F., I.F.D., I.G., J.A.C., M.G.C., M.L., M.M., P.T., R.A.S., R.F., S.F., S.J.S., S.O., S.Y.K. and W.C.H. analysed data. R.F., A.J.B., R.A.S. and W.C.H. wrote the manuscript.

Author Information The SNP data can be found at http://research3.dfci.harvard.edu/cdk8colon/index.php. Reprints and permissions information is available at www.nature.com/reprints. The authors declare competing financial interests: details accompany the full-text HTML version of the paper at www.nature.com/nature. Correspondence and requests for materials should be addressed to W.C.H. (william_hahn@dfci.harvard.edu).

METHODS

Plasmids and antibodies. Human CDK8 and CDK8 (D173A) kinase-dead mutants were obtained in a pCMV2 vector backbone (gift from A. Rice) and cloned into a pBABE-puro-Flag retroviral vector. pBABE-puro-S33Y-β-catenin has been previously described[1]. pTOPFLASH and pFOPFLASH were generated by inserting a tandem 8× TOPFLASH or 8× FOPLFASH element into vectors pGL4.15 (Promega) and pGL4.80 (Promega), respectively. The following antibodies were used for all experiments: CDK8 (goat anti-hCDK8, Santa Cruz, sc-1521); MYC (rabbit anti-c-Myc, Santa Cruz, sc-788); p21 (rabbit anti-p21, Santa Cruz, sc-397); Notch (rabbit anti-Notch1, Epitomics, 1935); β-catenin (mouse anti-β-catenin, BD Transduction, 610154); β-actin (HRP conjugated β-actin; Santa Cruz, sc47778); goat IgG (Santa Cruz, sc-2028).

Cell culture and gene transfer. DLD-1, COLO-741, RKO, HCT116, SW-1463, T84, COLO-205, SW480, HT29, SW837 and HCA-7 cell lines were maintained in DMEM supplemented with 10% fetal bovine serum (Sigma). FHC cells were grown in DMEM and F12 medium (ATCC) supplemented with 10% fetal bovine serum (Sigma). All transfections were performed using Fugene 6 (Roche) and retroviral and lentiviral infections were performed as described[30]. To generate stable cell lines, cells were selected using 2 μg ml^{-1} of puromycin for 3 days, 200 μg ml^{-1} of hygromycin for 5 days or 500 μg ml^{-1} of G418 for 7 days. Clonal DLD-1^{Rep} and SW480^{Rep} cells were generated by serial transfection of DLD-1 and SW480 cells with the pTOPFLASH plasmid and pFOPFLASH plasmids, selection, and isolation of single cell clones after 14 days of growth in media with the appropriate resistance markers.

Focus formation, anchorage-independent growth and tumorigenicity assays. Anchorage-independent growth and tumorigenicity assays were performed as described[31]. Colonies from soft agar assays were assessed using images taken from plates examined under a dissection microscope and analysed using ImageJ software.

Expression analysis and chromatin immunoprecipitation. Immunoblotting was performed according to standard methods. For RT–PCR, total RNA was isolated using RNeasy (Qiagen) and cDNA synthesis was performed using the Advantage RT/PCR kit (Clontech). For quantitative RT–PCR, we used SYBR Green (Applied Biosystems). ChIP experiments were performed using EZ-CHIP (Upstate Biotech).

RNAi and reporter assays. Lentiviral infections of cell lines were performed using pLKO.1 lentiviral shRNA constructs generated by the RNAi consortium, which are listed in Supplementary Table 6. A pLKO.1 *shGFP* control plasmid containing the following targeting sequence was used: GCCCGCAAGCTGAC CCTGAAGTTCATTCAAGAGATGAACTTCAGGGTCAGCTTGCTTTTT. For high-throughput screening, cells infected with lentiviruses were allowed to grow for 4 days (DLD-1^{Rep}) or 5 days (HCT116). Dual Glo Luciferase (Promega) and Cell Titer Glo (Promega) were used to measure luciferase activity for DLD-1^{Rep} β-catenin activity and HCT116 proliferation, respectively. *Z* scores were calculated as described[32]. Suppression of β-catenin activity was defined as the ability of at least one shRNA to decrease activity more than 2 s.d. below the *Z* score. Suppression of proliferation was defined as the capacity for at least one shRNA to decrease proliferation more than 1.5 s.d. below the mean *Z* score and at least one extra shRNA targeting the same kinase to decrease proliferation more that 1 s.d. below the mean *Z* score. RNAi analysis of resident 13q12 genes essential for colon cancer proliferation was conducted using colon cancer cell lines that harbour (HT29 and COLO-205) or lack (SW837 and T84) chromosome 13 copy number gain. These cells were infected in parallel with lentiviruses harbouring at least three different shRNA targeting each gene and subsequent cell proliferation and gene expression was measured. shGSEA analysis was then conducted to derive a statistical score of proliferative effects.

Tumour collection, processing and SNP array analysis. DNA was collected from freshly frozen colorectal adenocarcinomas derived from primary resection specimens (123) and matched normal colonic tissues (115). Whenever possible, samples with estimated tumour content of more than 70% on the basis of pathological review were chosen for this analysis. Samples were obtained from the following five sites: the Massachusetts General Hospital (25), the Brigham and Women's Hospital/Dana Farber Cancer Institute (46), the Vall d'Hebron Hospital in Barcelona (9) and the Genomics Collaborative Incorporated (43). From each sample, 250 ng of unamplified DNA was digested with StyI (New England Biolabs), ligated to a universal adaptor using T4 ligase (New England Biolabs) and PCR amplified using Titanium Taq (Clontech). Using Affymetrix protocols, amplified DNA underwent clean-up steps and was then fragmented with DNaseI (Affymetrix), labelled, denatured and hybridized to the StyI chip of the Affymetrix 500K Human Mapping Array set. The GeneChip Scanner 3000 7G (Affymetrix) was used to scan all hybridized arrays. Samples were run in 96-sample batches which included 6 standard control DNA input samples as well as DNA from tumours and normal tissues of different histologies. Raw array data from samples in each batch were processed using the GenePattern software package (http://www.broad.mit.edu/cancer/software/genepattern/) to create copy number values for each SNP. All mapping information for each SNP, cytoband and gene was on the basis of Affymetrix annotations and the hg17 (May 2004) genome build (http://genome.ucsc.edu). See Supplementary Methods for further details.

Immunohistochemical analysis and fluorescence *in-situ* hybridization (FISH). A TMA composed of 88 cases of human colon cancer tissue and matching patient normal colon was obtained from the Center for Molecular Oncologic Pathology (Brigham and Women's Hospital/Dana Farber Cancer Institute). Thirty-eight cases were excluded on the basis of inadequate or insufficient tissue for comparative analysis with FISH. The TMAs were subjected to citrate buffer and pressure cooker antigen retrieval and immunostained with the CDK8 antibody (1:100 dilution). Tissues were assessed for morphology and expression by a pathologist (S.F.). Four-micrometre TMA sections were mounted on standard glass slides for FISH. BAC clones were hybridized in dual colours as follows: the RB1 probe (Abbott Molecular/Vysis) was used as a chromosome 13 control probe and was obtained labelled in SpectrumGreen, and the RP11-726I20 BAC, spanning *CDK8*, was labelled with SpectrumOrange dUTP (Abbott Molecular/Vysis).

Statistical analysis of genomic copy number alterations and GISTIC. In brief, raw SNP data are renormalized by comparison of the SNP signal intensity in the tumour to that of the average of the five closest normal samples. Renormalized data are then smoothed and segmented and corrected for batch effects and published copy number polymorphisms. To identify recurrent amplifications above a threshold $\log_2$ copy number of 0.1, each locus is first given a score on the basis of frequency and intensity of amplification. To determine the false discovery rate (*q*-value), the score at each SNP is compared against that achieved by randomly permuting the copy number data. Detailed methods of the GISTIC algorithm are found elsewhere[31,33].

Derivation of GSEA for statistical analysis of shRNA effects. To analyse the screen results on a per-gene basis and compare the effects of genes between cell lines with 13q12 copy number gain and deletion, we adapted the GSEA method to evaluate enrichment of gene sets in gene expression analysis[14]. In analogy with GSEA, the 'gene set' is the set of shRNA hairpins that represent a gene. We first standardize each sample shRNA construct value using the median and maximum absolute deviation of a collection of shRNA control hairpins expressed in the same cell line. The hairpins were then mapped across samples and sorted according to the magnitude of their differential cell proliferation score (that is, the difference of their means in each phenotype: 13q12 copy number gain versus control). Only shRNAs that suppressed their target gene by more than 70% were analysed for cell proliferation effects. Once the effective hairpins have been sorted this way, an 'enrichment' score is computed for each gene on the basis of the distribution of the shRNAs in the list. The enrichment score is computed using a two-sample statistic on the basis of the likelihood ratio and is representative of both the extremeness of the hairpins' differential cell proliferation scores of a given gene, and also of their consistency. The lower the hairpins of a given gene appear in the list, the higher their (negative) enrichment score and the more the decrease in proliferation the gene produces between the samples with and without 13q12 copy number gain. To account for the fact that different genes have different number of hairpins, we normalized each enrichment score using random permutations of a hairpin set of the same size. This permutation test also provides nominal *P* values for each gene-enrichment score. The end result is a list of genes sorted by their normalized enrichment scores and a set of complementary estimates of statistical significance such as nominal, family wise and Bonferroni *P* values plus a false discovery rate.

Fluorescence *in situ* hybridization. Four-micrometre TMA sections were mounted on standard glass slides and baked at 60 °C for at least 2 h. Slides were soaked for 15 min in xylene that was pre-warmed to 55 °C and then in room temperature xylene for a further 15 min. Slides were then soaked in two successive 100% ethanol washes for at least 2 min each and allowed to air dry. The slides were then boiled in 100 mM Tris, 50 mM EDTA, pH 7.0, for 1 h, and soaked in 2× standard sodium citrate (SSC) for 5 min. Slides were placed on a 37 °C Thermobrite (StatSpin) and digested with Digest-All III solution (Invitrogen) for two 15 min digestions. Samples were fixed for 2 min in 10% phosphate buffered formalin, followed by dehydration for 2 min each in 70%, 80%, 90% and 100% ethanol.

One microgram of BAC DNA was labelled using a nick-translation kit (Abbott Molecular/Vysis) following the manufacturer's instructions. Labelled DNA was precipitated at −80 °C for 1 h with 10 μl Cot-1 DNA (1 mg ml^{-1} stock), pelleted, air dried and resuspended in the appropriate volume of 50% Hybrisol (50% formamide, 2× SSC, 10% dextran sulphate). Resuspended pellets were incubated at 37 °C for 1 h. The final probe concentration was approximately 50–100 ng μl^{-1}.

Slides were denatured in Coplin jars with 70% formamide/2× SSC at 94 °C for 5 min, followed by successive 2 min dehydrations each in 70% and 80% ethanol, at 4 °C, and 90% and 100% ethanol, at room temperature. Probes were denatured at 94 °C for 10 min, followed by snap cooling on ice. Denatured probes were applied to air-dried slides; coverslips were applied and then sealed with rubber cement. Hybridizations were performed for at least 16 h at 37 °C in a dark humid chamber. Slides were washed in 2× SSC at 70 °C for 10 min, rinsed at room temperature in 2× SSC and counterstained with DAPI (4′,6-diamidino-2-phenylindole, Abbott Molecular/Vysis). Slides were imaged using an Olympus BX51 fluorescence microscope. Individual images were captured using an Applied Imaging system running CytoVision Genus version 3.9.

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