Supplemental Materials and Methods

Additional antibodies used that are not described in the manuscript

For western blot analysis of E2F2 – sc9967 and for western blot analysis of E2F3 – sc69683. Both antibodies were purchased from Santa Cruz Biotechnology.

Plasmids and shRNA cloning

Most of the shRNAs tested in the screen were obtained from Open Biosystems and were subsequently sub-cloned from their pSM2 plasmid into the pLM-puro plasmid that we prepared. The "V2HS_xxxxx" labeling for each shRNA obtained from Open Biosystems is used in the manuscript despite that each shRNA was subcloned from the pSM2 plasmid provided by Open Biosystems into the pLM-puro plasmid prior to use in the screen. This is meant to facilitate identification of the sequence of particular shRNAs that were used in the screen. However, recently Open Biosystems switched the backbone plasmid used for shRNA expression and so they now list these plasmids as "V2LHS_xxxxx". We have retained the original naming of the plasmids since this is what we purchased but the shRNA sequence in each plasmid may be obtained from the Open Biosystems website by by using "V2LHS" rather than "V2HS" when searching their website.

The pLM-puro plasmid was constructed by first inserting an MluI site 3' to the EcoRI site of pMSCV (Clontech). Then the SalI-to-MfeI cassette encoding Mir30 miRNA flanking sequences was subcloned from pSM2 (Open Biosystems) into the XhoI/EcoRI

sites of pMSCV. This destroyed the XhoI and EcoRI restriction sites in pMSCV however the SalI-MfeI fragment subcloned from pSM2 also encodes XhoI and EcoRI sites that are used for insertion of an shRNA. Then, for all shRNA's obtained from Open Biosystems XhoI-to-MluI fragments encoding the shRNAs and downstream barcode sequences were subcloned from the corresponding pSM2 plasmids into pLM-puro.

Preparation of pLM-puro plasmids encoding shRNAs not purchased from Open Biosystems were prepared by ligating the following inserts containing the shRNAs in the XhoI/EcoRI sites of pLM-puro (reverse complement to siRNA guide sequence is underlined):

EBNA1mi1666 Insert:

CTCGAGAAGGTATATTGCTGTTGACAGTGAGCGCGTCCATTGTCTGTTATTTC
ATTAGTGAAGCCACAGATGTAATGAAATAACAGACAATGGACTTGCCTACTG
CCTCGGAATTC

Ff-Luciferasemi203:

CTCGAGAAGGTATATTGCTGTTGACAGTGAGCGCCGATATGGGCTGAATACA
AATTAGTGAAGCCACAGATGTAATTTGTATTCAGCCCATATCGTTGCCTACTG
CCTCGGAATTC

ORC1mi550:

CTCGAGAAGGTATATTGCTGTTGACAGTGAGCGCGGAAATATTCTGGTATGA
TTATAGTGAAGCCACAGATGTATAATCATACCAGAATATTTCCTTGCCTACTG
CCTCGGAATTC

ORC2mi1903:

CTCGAGAAGGTATATTGCTGTTGACAGTGAGCGAGGAACTGATGGAGTAGAG

TATTAGTGAAGCCACAGATGTAATACTCTACTCCATCAGTTCCCTGCCTACTG

CCTCGGAATTC

ORC2mi1981:

CTCGAGAAGGTATATTGCTGTTGACAGTGAGCGAGAGGCTTGAAGCTTTCCTT

TATAGTGAAGCCACAGATGTATAAAGGAAAGCTTCAAGCCTCCTGCCTACTG

CCTCGGAATTC

DDX5mi475:

CTCGAGAAGGTATATTGCTGTTGACAGTGAGCGACCTGCAAATGTCATGGAT

GTTTAGTGAAGCCACAGATGTAAACATCCATGACATTTGCAGGGTGCCTACT

GCCTCGGAATTC

DDX5mi2053:

CTCGAGAAGGTATATTGCTGTTGACAGTGAGCGCGCTCTTTATATTGTGTGTT

ATTAGTGAAGCCACAGATGTAATAACACACAATATAAAGAGCATGCCTACTG

CCTCGGAATTC

DDX5mi1314:

CTCGAGAAGGTATATTGCTGTTGACAGTGAGCGATGGGTTCTAAATGAATTC

AAATAGTGAAGCCACAGATGTATTTGAATTCATTTAGAACCCAGTGCCTACT

GCCTCGGAATTC

DDX5mi2008:

CTCGAGAAGGTATATTGCTGTTGACAGTGAGCGCCCCAATAAGACTTTAGAA

GTATAGTGAAGCCACAGATGTATACTTCTAAAGTCTTATTGGGATGCCTACTG

CCTCGGAATTC

DDX5mi960:

CTCGAGAAGGTATATTGCTGTTGACAGTGAGCGAAAGATTGTGGATCAAATAAGATAGTGAAGCCACAGATGTATCTTATTTGATCCACAATCTTCTGCCTACTGCCTCGGAATTC

Preparation of retrovirus and infections

For infection of HCT116 cells, Phoenix-Amphotrophic packaging cultures seeded 24hrs prior with 1.4 million cells per well in 6-well tissue culture plates were co-transfected with pLM-puro encoding an shRNA and pVSVG plasmids using lipofectamine 2000 (Invitrogen #11668-027) following the manufacturers protocol. 24hrs after transfection, the transfection media on each culture was replaced with 2mL DMEM + 10% FBS. At both 36hrs and 48hrs post-transfection viral supernatant was collected from each packaging culture, filtered through a 0.45micron filter (Corning #431220), and supplemented with 8µg/mL hexadimethrine bromide (Sigma H-9268). Then 0.5mL viral supernatant was added to a well of a 24-well tissue culture plate containing HCT116 cells seeded 24hrs prior with 150,000 cells per well. Thus each HCT116 culture was infected with the 36hr and 48hr viral supernatant for a total of two rounds of infection. At 24hrs after the second round of infection, each infected HCT116 culture was suspended and seeded into a well of a 6-well tissue culture plate in DMEM + 10% FBS + 1.5µg/mL puromycin. Cultures were selected for 4 days then cells were collected for experiments.

Retrovirus packaging and infection of the different breast cancer cell lines was performed as described above with the following exceptions. MCF10A cells were infected with viral supernatant diluted 1-to-2 with MCF10A growth media (see cell lines and growth media above). All breast cancer cell lines were seeded in 6-well tissue culture plates 24hrs prior to infection (except ZR-75-1 and MDA-MB-453 cells which were seeded to 6-well tissue culture plates 48hrs prior to infection). Infected cultures were selected with 2-to-3μg/mL puromycin for 4-to-5 days.

Colony Formation Assays

24hrs after p220.2 plasmid transfection, HCT116 cells from each transfected culture were suspended by trypsinization and counted using a hemacytometer. For the proliferation assay, one well of a 12-well tissue culture (TC) plate was seeded with 5,000 cells per suspension in 1mL DMEM + 10% FBS + 1.5μg/mL puromycin. For plasmid stability assay, the same cell suspensions used for seeding the proliferation assay cultures were also used to seed 50,000 cells per suspension to one well of a 12-well TC plate each seeded in 1mL DMEM + 10% FBS + 1.5μg/mL puromycin + 400μg/mL hygromycin. Selection media was replaced on each culture every 3rd day. Colonies in proliferation assay cultures were expanded for 8 days and plasmid stability assay cultures for 10 days prior to staining. For staining, 1/10 volume of Formaldehyde solution (Mallinckrodt #5014-04) was added to each culture and cultures were incubated at 4^{0} C for 30 minutes. The fixing media was then removed and each well was washed once with PBS then once with distilled water. After aspirating the final wash from each well these were stained with 0.5% crystal violet for 30 minutes. After staining, excess stain was removed by

gently rinsing the wells with distilled water. The amount of dye taken up by colonies in each well was quantitated by destaining each well with 1mL 10% glacial acetic acid for 30 minutes and measuring the absorbance of the destainate at OD 595 on an Eppendorf Biophotometer spectrophotometer.

The effect of each experimental shRNA tested in the screen on cell proliferation was determined by dividing the colony formation result (measured absorbance – see above) for the culture transduced with the experimental shRNA by the colony formation result for the negative control culture transduced with the Ff-luciferasemi203 shRNA where colonies for both cultures were expanded in media containing puromycin only. The effect of each experimental shRNA on plasmid stability was determined by dividing the colony formation result for the culture transduced with the experimental shRNA by the colony formation result for the negative control culture transduced with the Ff-luciferasemi203 shRNA where the colonies for both these cultures were expanded in media containing both puromycin and hygromycin. The plasmid stability ratio for the experimental shRNA was then calculated by dividing the plasmid stability result over the proliferation assay result obtained as described above. Thus, a plasmid stability ratio of 1 indicates that the experimental shRNA did not impair plasmid stability greater than cell proliferation and a plasmid stability ratio of less than 1 indicates that the experimental shRNA did impair plasmid stability greater than cell proliferation.

For breast cancer colony formation assays, following selection of infected cells with puromycin the cells from each culture were suspended by trypsinization, counted using a

hemacytometer, then seeded to 12-well TC plates for colony formation in 1mL appropriate growth media (see cell lines and growth media above) including 2-to-3μg/mL puromycin per well. Cultures were seeded for colony formation with the following number of cells 2,000 cells (MCF10A), 10,000 cells (Hs578t, SK-BR-3, EVSA-T, and HCC1143), or 20,000 cells (EFM-19, MDA-MB-453, and ZR-75-1). When cultures transduced with the negative control EBNA1mi1666 shRNA neared confluency they were stained with crystal violet and quantitated as described above.

Percent inhibition of colony formation was calculated for MDA-MB-453 cells transduced with either the DDX5mi2008 shRNA or Empty vector (virus not encoding an shRNA) and expanded in increasing concentration of trastuzumab using the equation, $(1 - A_N / A_0) \times 100$ where A_N is the measured absorbance for either the DDX5mi2008 or Empty vector transduced cell cultures grown in media supplemented with a specific concentration of trastuzumab "N" and A_0 is the measured absorbance of either the DDX5mi2008 or Empty vector transduced cell cultures grown in media without trastuzumab.

SiRNAs sequences and transfections

DDX5si2008: CCCAAUAAGACUUUAGAAGUA

DDX5si2053: GCUCUUUAUAUUGUGUGUUAU

EBNAsi1666: GUCCAUUGUCUGUUAUUUCAU

E2F1si1533: CUCCCAGCCUGUUUGGAAA

E2F2si809: GACUCGGUAUGACACUUCG

E2F3si1041: CGUCCAAUGGAUGGGCUGC

All siRNAs were ordered from Dharmacon with dTdT 3' overhangs where the DDX5si2008, DDX5si2053, and EBNA1si1666 siRNAs are ON-TARGETplus – Specificity Enhanced modified. HCT116 cells were seeded into 6-well TC plates at 125,000 cells per well in 2mL DMEM + 10% FBS. 24hrs after seeding, cultures were transfected with 10nM siRNA and 5µL Lipofectamine RNAiMAX (Invitrogen catalog number 13778-075) prepared in 0.5mL Opti-Mem (Gibco catalog number 31985-062) as per the manufacturer's instructions then added to each HCT116 culture growing in 2mL DMEM + 10% FBS.

Cell cycle and BrdU incorporation flow cytometry analysis

HCT116 cells growing in 6-well TC plates and transfected with the indicated siRNA for either 24hrs or 48hrs as described above were collected by trypsinization, washed twice with PBS, then incubated overnight in 70% ethanol at -20^0 C. The cells were then washed twice with PBS then incubated in PBS + 20µg/mL propidium iodide + 20µg/mL RNase A for 20 minutes at 37^0 C. Flow cytometry analysis was performed on a Becton Dickinson LSR-II cytometer using FACSDiva software.

For BrdU incorporation experiments, HCT116 cells growing in 6-well TC plates and transfected with the indicated siRNA for 48hrs were pulsed with 10µM BrdU for 10 minutes. BrdU containing media was then aspirated and cells were suspended by

trypsinization, washed in PBS, and solubalized in 70% ethanol as described above. The following day, the cells were processed and stained with FITC-conjugated anti-BrdU (Becton Dickinson #347583) using the manufacturer's protocol. Flow cytometry analysis was performed on a Becton Dickinson LSR-II cytometer using FACSDiva software.

Quantitative PCR analysis

RNA was prepared from siRNA transfected HCT116 cultures at the indicated timepoints post-siRNA transfection using the RNeasy Mini Kit (Qiagen cat. # 74104) including on-column DNase digestion (Qiagen cat. # 79254) and eluted in the supplied RNase-free water. RNA from SK-BR-3 cultures was prepared from cells 8 days post-shRNA transduction. The cDNA used for Q-PCR was prepared from 1µg each RNA sample using TaqMan Reverse Transcription Reagents (Applied Biosystems #N808-0234) with random hexamer priming in a GeneAmp PCR system 9700 thermocycler. Each Q-PCR reaction was prepared using 2µL of 1-to-20 diluted cDNA and 13µL LightCycler 480 SYBR Green I Master Mix (Roche #04887352001) and were performed in 384-well plates using the LightCycler 480 (Roche) as per manufacturer's instructions. Primer sequences used for analysis of cDNA samples are listed below:

MCM3-128F: accaataccggctgattgtc, MCM3-217R: gctcctcaaaggcattgttc, MCM5-367F: gacatccaggtcatgctcaa, MCM5-456R: agggatcttcaccaggtgtg, CDC6-633F: cttaagccggattctgcaag, CDC6-715R: cagtcctcaaggacatgcaa, POLA2-634F: aagctcccagacattcgaga, POLA2-736R: gggctagcaaaggagtgaaa, CDC45-969F:

gcaggtgaagcagaagttcc, CDC45-1069R: gcatgtccttcatcccaaat, MCM10-545F: cgctaccaagaaccaagagg, MCM10-632R: ggtgcacttgtcatccttga, GAPDH-F: cctgacctgccgtctagaaa, GAPDH-R: ctccgacgcctgcttcac, ACTIN-452F: ttcaacaccccagccatgt, and ACTIN-523R-gccagtggtacggccaga.

Q-PCR analysis of ChIP samples was performed using 2μL of the samples that were each diluted 1-to-3 with nuclease-free water. 13μL LightCycler 480 SYBR Green I Master Mix (Roche #04887352001) was added to each ChIP sample and PCR reactions were performed in 384-well plates using a LightCycler 480 (Roche) as per manufacturer's instructions. Primer sequences are listed below.

CDC6pro-F: gtttgttcaggggcttgtg, CDC6pro-R: cgagcaatcctcttctttcc, CDC45pro-F: agaaaggaaggctgggaact, CDC45pro-R: aagactcccgccaaatca, MCM5pro-F: ttccttcccagccagaagt, MCM5-R: agatgattggctgcaaagtg, GAPDHpro-F: caattccccatctcagtcgt, GAPDHpro-R: agcaggacactagggagtcaa, CD4pro-F: tgtgctctgcccagttgtct, CD4pro-R: gctcatgaccagttccaagagaa, SimpleChIP Gamma Actin promoter primers were ordered from Cell Signaling cat. # 5037

Differential expression, Gene set, transcription factor motif, and TCGA database gene expression analysis

Differential expression analysis for individual genes and gene sets was performed as follows. We inferred individual genes or gene sets differentially expressed between the DDX5 knockdown groups (DDX5si2008 and DDX5si2053) vs. the control groups

(EBNA1si1666 and Mock). Gene set or pathway analysis was performed using generally applicable gene set enrichment (GAGE) (1). The most differentially regulated Gene Ontology groups were selected with FDR q-value < 0.1. Individual gene analysis selected differentially regulated genes with FDR q-value < 0.1 using a nonparametric rank test based on GAGE.

We designed the following procedure for our transcription factor motif analysis. We identify transcription factor target gene sets whose expression levels are significantly perturbed as a whole using GAGE (1). These gene sets are collected from Misa published work (2), deposited in the Molecular Signatures Database (MSigDB). This result gave us an idea of what are the potential transcription factors that co-regulate genes together with DDX5. We compared the results from the DDX5si2008 knockdown group with the EBNA1si1666 and mock transfected groups and only kept those transcription factor target gene sets that were significant. Transcription factor activities/functions are redundant, some closely related transcription factors share a very similar binding motif and regulate a similar set of target genes. We eliminated this redundancy by clustering similar transcription factor target gene sets together or kept the most representative transcription factor target gene set. We then extracted genes that are substantially perturbed in these representative transcription factor target sets. Finally, we did a promoter sequence analysis on these genes to confirm that their promoters have the binding motif for the transcription factor. MSCAN was applied to search for the transcription factor binding motifs from JASPAR database (3).

Gene expression data for 533 breast tumors was obtained from the TCGA database (http://cancergenome.nih.gov/). Pearson correlations were calculated to identify genes that were correlated with DDX5 expression in the tumors. To identify Reactome pathways enriched for genes correlated with DDX5 we performed the following calculation. Since there are 3788 genes in the Reactome pathways that we could find expression results for we set a threshold for the Pearson p-value for each gene at 1.32×10^{-5} (corresponding to a p-value of 0.05 divided by 3788 genes). Among these 3788 genes we identified all those whose expression was correlated with DDX5 with this p-value or less. We then used Fisher's exact test to identify Reactome pathways that were enriched for genes correlated in expression with DDX5 with a p-value at or below this threshold.

Nuclear extract preparation and immunoprecipitation assays

For assays testing asynchronous cultures, 8×10^8 HCT116 cells were collected from 10cm plates each at ~50% confluency. Cells were washed first with PBS then with 4mL buffer A (10mM Tris pH7.5, 5mM NaCl, 2mM $MgCl_2$, 0.5mM DTT, Roche protease inhibitor cocktail #11873580001, and Roche phosphatase inhibitor cocktail #04906845001). The cells were then lysed in 4mL buffer A + 0.5% NP40 for 10 minutes on ice and nuclei pelleted in a Beckman GS-6R centrifuge at 1,500rpm for 5 minutes at 4^0 C. Nuclei were suspended in 2mL buffer A + 2mM $CaCl_2$ and incubated at 37^0 C for 1 minute after which micrococcal nuclease (Sigma N3755) was added to a final concentration of 3.2 units / mL and the nuclei were further incubated at 37^0 C for 10

minutes. Digestion was stopped by adding EGTA to 4mM and placing the suspension on ice. The suspension was then centrifuged 2,000rpm for 10 minutes in the Beckman GS-6R centrifuge and the resulting pellet suspended in 2mL buffer B (10mM Tris pH7.5, 400mM NaCl, 2mM $MgCl_2$, 10% glycerol, 2mM EGTA, 0.5mM DTT, protease inhibitor cocktail and phosphatase inhibitor cocktail). The extracts were then rotated 1hr at 4^0 C then centrifuged in a Sorvall SS-34 rotor using a Sorvall RC-5C Plus centrifuge at 10,000rpm for 10 minutes at 4^0 C. The NaCl concentration in the resulting supernatant was then adjusted to 150mM using buffer C (10mM Tris pH7.5, 2mM $MgCl_2$, 10% glycerol, 2mM EGTA, 0.5mM DTT, protease inhibitor cocktail and phosphatase inhibitor cocktail) and the centrifugation was repeated to clear away precipitated material. The resulting nuclear extract was pre-cleared with GammaBind G Sepharose (GE Healthcare #17-0885-01) pre-equilibrated in buffer D (10mM Tris pH7.5, 150mM NaCl, 2mM $MgCl_2$, 10% glycerol, 2mM EGTA, 0.5mM DTT, protease inhibitor cocktail and phosphatase inhibitor cocktail) and the protein concentration in the nuclear extract was measured using Bradford Assay.

For immunoprecipitation assay 0.5mg freshly prepared and pre-cleared nuclear extract was incubated with either 5µg rabbit anti-DDX5 (Bethyl laboratories #A300-523A) or 5µg normal rabbit IgG (Caltag laboratories #10500C) while rotating overnight at 4^0 C. The resulting solutions containing immunocomplexes were then coupled for 1hr while rotating at 4^0 C with GammaBind G Sepharose (GE Healthcare #17-0885-01) pre-equilibrated in buffer D. The beads were washed 3 times with buffer D then suspended in loading dye for western blot analysis.

GST-E2F1 and DDX5 cloning, purification, and interaction experiment

The pSG5L HAE2F1 plasmid (plasmid 10736) was obtained from Addgene (4). E2F1 was then amplified using this plasmid as template, the forward primer: actgggatccatggccttggccgggg, and the reverse primer: actgctcgagtcagaaatccaggggggt. The resulting PCR product was digested with BamHI and XhoI and ligated into the pGEX-6p-1 plasmid. The pCMV6-XL4 DDX5 plasmid was obtained from OriGene (cat. # sc125285). DDX5 was amplified using this plasmid as template, the forward primer: ACTGAAGCTTatgtcgggttattcgagtg, and the reverse primer: ACTGCTCGAGttattgggaatatcctgttg. The resulting PCR product was digested with HindIII and XhoI and ligated into the pLPC vector that encodes the T7 promoter upstream of the multiple cloning site. Both the E2F1 and DDX5 inserts were sequenced in their respective plasmids to verify that the correct sequences were cloned.

Expression of GST-E2F1 was induced in E. coli with 0.5mM IPTG and allowed to shake overnight at 16^0C. Bacteria were lysed in buffer containing 50mM Tris pH 7.5, 100mM NaCl, 5mM $MgCl_2$, 10% glycerol, 1% Triton X-100, 1mM PMSF, 5mM Benzamidine-HCl, 1mM DTT, complete Roche protease inhibitor cocktail EDTA-free (cat. #11-873-580-001), and 200mg/mL Lysozyme. Lysis was performed for 1hr with rotation at 4^0C followed by sonication for 3 minutes on ice for cycles of 10 seconds on / 10 seconds off using a Tekmar TMX 600 sonicator set at 30% amplitude. Soluble lysate was then separated from insoluble material by centrifugation at 12,000rpm for 45 minutes in a

Sorvall SS-34 rotor. Soluble GST-E2F1 was then coupled to glutathione sepharose beads (GE Healthcare 17-5132-01) for 2hrs with rotation at 4^0C where the beads were pre-equilibrated to lysis buffer prior to incubation with protein. The GST-E2F1 coupled glutathione beads were then loaded into an Econo-Pac chromatography column (Bio-Rad catalogue number 732-1010) and extensively washed with 50 volumes of lysis buffer containing 500mM NaCl. GST-E2F1 coupled glutathione beads were then suspended in buffer containing 50mM Tris pH 7.5, 100mM NaCl, and complete Roche protease inhibitor cocktail EDTA-free and the amount of GST-E2F1 bound to the beads was determined by Coomassie SDS PAGE analysis using a titration of bovine serum albumin as a reference.

S^{35}-methionine radiolabeled DDX5 was prepared using 2µg pLPC-DDX5 plasmid and the Promega TnT Quick coupled Transcription/Translation Kit and the manufacturer's instructions. Glutathione sepharose beads previously conjugated to 5µg of either GST or GST-E2F1 were equilibrated to binding buffer containing 25mM Tris pH 7.5, 75mM KCl, 0.1mM EDTA, 5mM $MgCl_2$, 10% glycerol, 1mM DTT, 0.05% NP40, and Roche complete protease inhibitor cocktail minus EDTA. 10µL radiolabeled DDX5 was diluted with 390µL binding buffer then added to either the GST-E2F1 or GST coupled glutathione sepharose beads and rotated for 2hrs at 4^0C. The glutathione sepharose beads were then washed with binding buffer and suspended in loading dye for SDS-PAGE analysis. Radiolabeled DDX5 bound to either the GST or GST-E2F1 conjugated glutathione sepharose beads was detected using a BAS IIIS phosphoimager screen and Fujifilm FLA-5100 phosphoimager.

Chromatin Immunoprecipitation

HCT116 cultures were seeded with 125,000 cells per well in 6-well TC plates and transfected with the indicated siRNAs as described above. 48hrs post-siRNA transfection 2.5 x 10^7 cells per siRNA condition were harvested by trypsinization and fixed with 1% formaldehyde for 10 minutes at room temperature. Fixing reactions were each quenched by addition of glycine to 125mM and incubation for 5 minutes at room temperature. Cell pellets were washed with PBS then lysed for 10 minutes on ice in buffer containing 10mM Tris pH8.0, 10mM NaCl, 0.2% NP40, Roche EDTA-free protease inhibitor cocktail cat. # 11873580001, and Roche PhosphoSTOP cocktail cat. # 04906845001. Nuclei were pelleted in an eppendorf 5430 microcentrifuge at 4^0 C for 30 seconds then each suspended in 1mL buffer containing 50mM Tris pH8.0, 10mM EDTA, 1% SDS, protease/phosphatase inhibitors with rotation for 20 minutes at 4^0 C. Lysates were diluted to 2mL with dilution buffer (20mM Tris pH8.0, 2mM EDTA, 150mM NaCl, 0.01% SDS, 1% Triton X-100, and protease/phosphatase inhibitors) then sonicated with a Bioruptor UCD-200 at 4^0 C set at medium power for 8 cycles of 30 seconds on and 30 seconds off. Samples were clarified by centrifugation in a microcentrifuge at 4^0 C for 5 minutes. Supernatants were pre-cleared for 2hrs at 4^0 C with 35µL of a 50:50 slurry of Gammabind G Sepharose (GE Healthcare cat. # 17-0885-01) in dilution buffer. Antibodies were also coupled to 35µL of a 50:50 slurry Gammabind G Sepharose beads in PBS for 2hrs at 4^0 C (5µg each antibody used for ChIP). Pre-cleared nuclear extracts were further diluted to 4mL with Dilution buffer then 1mL per extract was incubated with

antibody-coupled beads overnight with rotation at 4^0C. 0.2mL pre-cleared nuclear extract was kept for use as input for Q-PCR analysis.

Following immunoprecipitation the beads were washed once with low salt buffer (20mM Tris pH8.0, 150mM NaCl, 2mM EDTA, 0.1% SDS, 1% Triton X-100, protease inhibitor cocktail), twice with high salt buffer (low salt buffer + 500mM NaCl), once with Lithium Chloride buffer (10mM Tris pH8.0, 250mM LiCl, 1mM EDTA, 1% deoxycholate, and 1% NP40), then twice with 10mM Tris pH8.0, 1mM EDTA buffer. Protein-DNA complexes were eluted from the beads in 0.1M sodium bicarbonate, 1% SDS at room temperature. Crosslinks in the ChIP and input samples were reversed overnight by addition of NaCl to 300mM to each sample as well as 1µL RNase A (Sigma cat. # R4642) and incubation at 65^0C. ChIP and input samples were then incubated with 0.3µg/µL proteinase K for 2hrs at 42^0 C followed by either 1 round of phenol:chloroform (ChIP samples) or 2 rounds phenol:chloroform extraction (input samples) and 1 round of chloroform extraction. DNA was precipitated by ethanol precipitation then diluted in 60µL nuclease-free water for Q-PCR analysis.

Note that for the DDX5 ChIP results presented in Figure 5B HCT116 cultures were first serum starved for 48hrs then released by addition of serum to the media to 10% for 2hrs prior to cell harvesting since DDX5 ChIP signals were stronger under this condition where the cells are synchronously proceeding through the cell cycle than in asynchronous HCT116 cultures. For ChIP results presented in Figures 5C, D, E, and F asynchronous HCT116 cultures were used as described above. For SK-BR-3 ChIPs the samples were

processed as described above except they were prepared from 5 x 10^6 SK-BR-3 cells per shRNA and at 9 days after transduction with the indicated shRNAs. Primer sequences used for Q-PCR analysis are listed above.

References for supplemental materials and methods

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3. Alkema WB, Johansson O, Lagergren J, Wasserman WW. MSCAN: identification of functional clusters of transcription factor binding sites. Nucleic Acids Res 2004; 32: W195-8.
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