Supplemental Figure Legend:

Supplemental Figure 1.

Plasmid stability assay results and western blot analysis of cells transduced with the indicated shRNAs that target the expression of either (A) ORC2, (B) CDC6, (C) ORC3, or (D) CDC45. Blue bars on the plots show plasmid stability ratios for cells transduced with the indicated shRNAs. The black horizontal bar indicates plasmid stabiliy ratio of 0.45. PCNA is blotted as loading control for the different blots shown except the ORC2 blot where cross-hybridizing bands as indicated by an asterisk are used for loading controls. The lower bands on the western blots in (B) and (D) are CDC6 and CDC45 respectively.

Supplemental Figure 2.

(A) Plasmid stability assay results and western blot analysis of cells transduced with shRNAs targeting the expression of either (A) AND-1, (B) DDX5, or (C) NCAP-G2. Blue bars on the plots indicate plasmid stability results for cells transduced with the indicated shRNAs. PCNA is blotted as loading control for the different blots.

Supplemental Figure 3.

(A) Plasmid stability assay results for cells transduced with shRNAs targeting the expression of DDX17 (indicated as blue bars on the plot). (B) Western blot analysis of DDX17 and Beta-actin (loading control) in cells transduced with the indicated shRNAs. The p72 and p82 isoforms of DDX17 are detected on the blot.

Supplemental Figure 4.

(A) Flow cytometry analysis of cell cycle at 48hr post-transfection of cells with either DDX5si2053 (left panel) or EBNA1si1666 (right panel). (B) Analysis of BrdU incorporation at 48hr post-transfection of cells with either DDX5si2008, DDX5si2053, or EBNA1si1666. The box shows the percent of BrdU positive cells out of the total population. Note the BrdU positive population indicated by the box is not the same as the number of cells in S-phase presented in the histograms in (A) and Figure 3B in the

manuscript since S-phase cells poorly incorporating BrdU lie below the box. The number in the box indicates the percent cells in the population efficiently incorporating BrdU.

Supplemental Figure 5.

RNAi complementation was attempted in HCT116 cells by first infecting them with virus encoding either RNAi resistant DDX5 (LPC-DDX5) or no transgene (LPC-Empty). The cells were then infected with virus encoding either of two different DDX5 shRNAs (DDX5mi2008 or DDX5mi2053) or the negative control Renilla luciferase (RLuc) shRNA. (A) Q-PCR analysis of the abundance of the DDX5 transcript in RNAi-resistant DDX5 transduced or empty vector infected cells +/- DDX5 knockdown. Note the reduced abundance of the DDX5 transcript in cells infected with empty vector then transduced with the DDX5 shRNAs compared to empty vector infected cells transduced with the control RLuc shRNA. In contrast, cells transduced with the RNAi resistant DDX5 transgene have 8-to-10 fold greater DDX5 transcript expression than the empty vector infected cells transduced with the RLuc control shRNA. Moreover, the exogenous DDX5 transcript is RNAi resistant since neither of the DDX5 shRNAs downregulated the abundance of the DDX5 transcript in LPC-DDX5 cells transduced with the DDX5 shRNAs compared to the RLuc control shRNA. (B) Analysis of GAPDH transcript in the indicated cell lines demonstrating equivalent input cDNA was used in the Q-PCR reactions presented in (A). (C) Western blot analysis of DDX5 in whole cell extracts (WCE) obtained from either LPC-DDX5 (D5) or LPC-Empty (E) infected cells +/- DDX5 knockdown. The triangle indicated above the lanes loaded with WCEs from LPC-DDX5 and LPC-Empty cells transduced with the control RLuc shRNA indicates that these lanes were loaded with either equivalent total protein as the DDX5 knockdown WCEs or were diluted 1-to-10 to quantify DDX5 knockdown on the blot. Note that DDX5 knockdown in LPC-Empty infected cells transduced with either DDX5mi2008 (D5mi2008) or DDX5mi2053 (D5mi2053) was ~90% since the signal for DDX5 in these lanes is equivalent to the signal for DDX5 in the 1-to-10 diluted control RNAi WCE. Also note that despite that the cells transduced with the RNAi resistant exogenous DDX5 had 8-to-10 fold increased RNAi resistant DDX5 transcript that overall DDX5 protein level was still reduced ~10-fold by DDX5mi2008 and DDX5mi2053 indicating poor

expression of the RNAi resistant protein in these cells. Similar difficulty in overexpressing the RNAi-resistant DDX5 protein were experienced regardless of whether the pLPC or pMSCV retroviral backbones were used for exogenous protein expression in either HCT116 or SK-BR-3 cells.

Supplemental Figure 6.

(A) Plot showing the average fraction of cells in G1, S, and G2/M phase for cultures transfected with the indicated siRNAs at 24hrs post-transfection determined from 3 independent experiments. Red bar corresponds to cultures transfected with DDX5si2008, Blue bar – DDX5si2053, Green bar – EBNA1si1666, and Black bar – Mock transfected. Error bars show standard deviation calculated from 3 independent experiments per siRNA. (B) Same as in "A" except cell cycle was analyzed at 48hrs post-transfection with the indicated siRNAs. (C) Western blot analysis of DDX5, RB, and Beta-Actin in three independently derived HCT116 whole cell extracts each prepared 24hrs after transfection with either DDX5si2008 (labeled as "D5") or EBNA1si1666 (labeled as "E").

Supplemental Figure 7.

Heatmap showing row-wise standardized expression level for genes with conserved E2F-binding sites in their promoters at 24hrs after transfection of cells with the indicated siRNAs.

Supplemental Figure 8.

Western blot analysis of E2F1, E2F2, E2F3, CDC6, CDC45, MCM5, and Beta actin (loading control) in cells transfected with the indicated siRNAs targeting either E2F1 (E1), E2F2 (E2), or E2F3 (E3), or EBNA1 (Con). CDC6 expression is dependent on E2F1, but neither CDC45 nor MCM5 expression are dependent on any particular E2F protein indicating that these promoters are under the redundant control of these E2F family members in this cell line.

Supplemental Figure 9.

(A) Colony formation assay results for duplicate MDA-MB-453 cultures transduced either with DDX5mi2008 (left pair of 12-well tissue culture plates) or Empty vector (right pair of 12-well tissue culture plates) then seeded in media containing increasing concentration of trastuzumab. Values shown in the upper plates indicate the concentration of trastuzumab (μg/mL) added to the culture. (B) Plot of absorbance values measured for destaining results of cultures shown in (A) where the red line corresponds to cells transduced with the DDX5mi2008 shRNA and the blue line corresponds to cells infected with empty vector (no shRNA). (C) Plot of absorbance results for either DDX5mi2008 or empty vector infected cultures expanded in media containing each concentration of trastuzumab normalized to the absorbance of the respective infected culture expanded in media without trastuzumab. Thus, results plotted in red indicate the amount proliferation of DDX5mi2008 transduced cells expanded in media containing the various concentrations of trastuzumab normalized to the proliferation of DDX5mi2008 transduced cells expanded in media lacking trastuzumab and the results plotted in blue indicate the amount of proliferation of empty vector infected cells expanded in media containing the various concentrations of trastuzumab normalized to the proliferation of empty vector infected cells expanded in media lacking trastuzumab. Note that the red and blue lines do not overlap indicating that the combination of DDX5 knockdown and trastuzumab treatment results in synergistic inhibition of MDA-MB-453 proliferation.

Supplemental Figure 10.

Plot of copy number variation for probes along chromosome 17 in the indicated breast cancer cell lines that either have DDX5 amplification (A) or lack DDX5 amplification (B). The DDX5 locus is indicated with the red line.

Supplemental Figure 11.

Western blot analysis of E2F1 and DDX5 in DDX5 and IgG IP samples obtained from MDA-MB-453, SK-BR-3, and MCF10A nuclear extracts.