Supplemental Data

Primers

The sequence of primer sets used in this study are:

For ChIP analysis

Primer sets used for *p107*, *E2F1*, *cyclin A* and *cdc25A* promoters and U_{2C} have been described (Takahashi et al., 2000 and Tyagi et al., 2007).

1. *Apaf1*
Forward GGGTGTGTTTATTTGCATAAGCGGGC

Reverse TCTGGACAGCGGAGCAGTCAAAT

2. *p73*

Forward TGCGACGGCTGCAGGTAGGA

Reverse TGTGTCGCCTTGTCCACTAGCTC

3. $p14^{ARF}$

Forward CGCTGAGGGTGGGAAGATGGT

Reverse GTTCCTCTCCCTCCCGCCTA

4. *p21*

Forward AGCTGCCGAAGTCAGTTCCTTGT

Reverse TCTCTCACCTCCTCTGAGTGCCT

For RT-PCR analysis

Primer sets used for *E2F1* and *GAPDH* mRNA have been described (Tyagi et al., 2007).

1. *Apaf1* mRNA

Forward TCCAGTCCAGGTTTCAGCACAAGA

Reverse TTCACTGTTTCCTGATGGCCTCGT

2. *p73* mRNA

Forward CGGGCCATGCCTGTTTACAAGAAA

Reverse TGGAGCAGACTGTCCTTCGTTGAA

3. *p14^{ARF}* mRNA

Forward AGGTTCTTGGTGACCCTCCGGATT

Reverse CCCATCATCATGACCTGGTCTTCT

4. *p21* mRNA

Forward TTAGCAGCGGAACAAGGAGTCAGA

Reverse ACACTAAGCACTTCAGTGCCTCCA

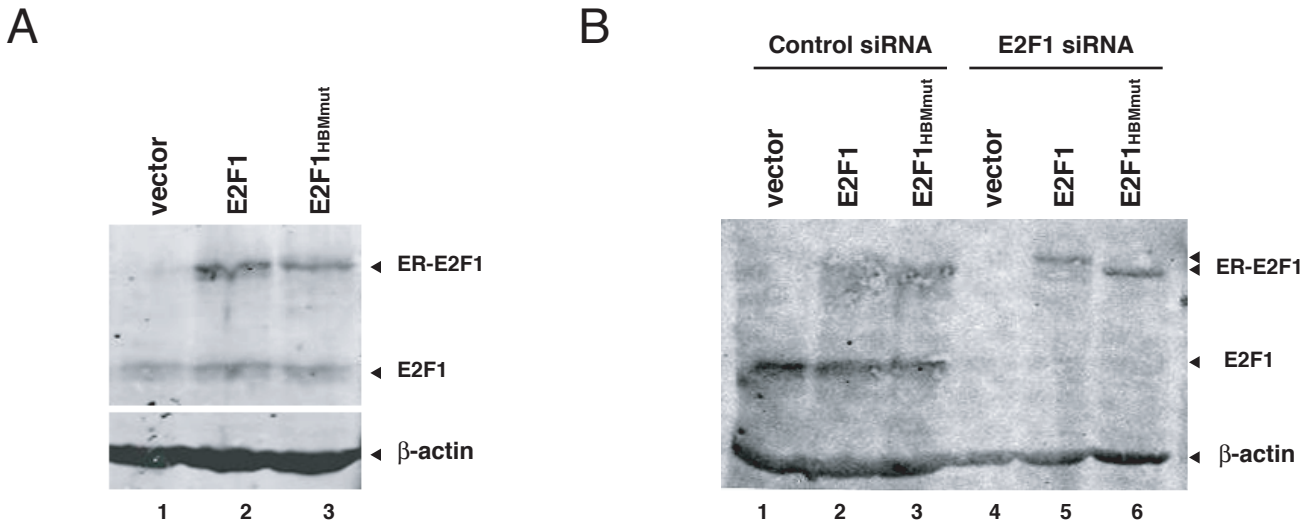
5. *hcf-1* mRNA

Forward AAGACAGCTCTGGCACCAA

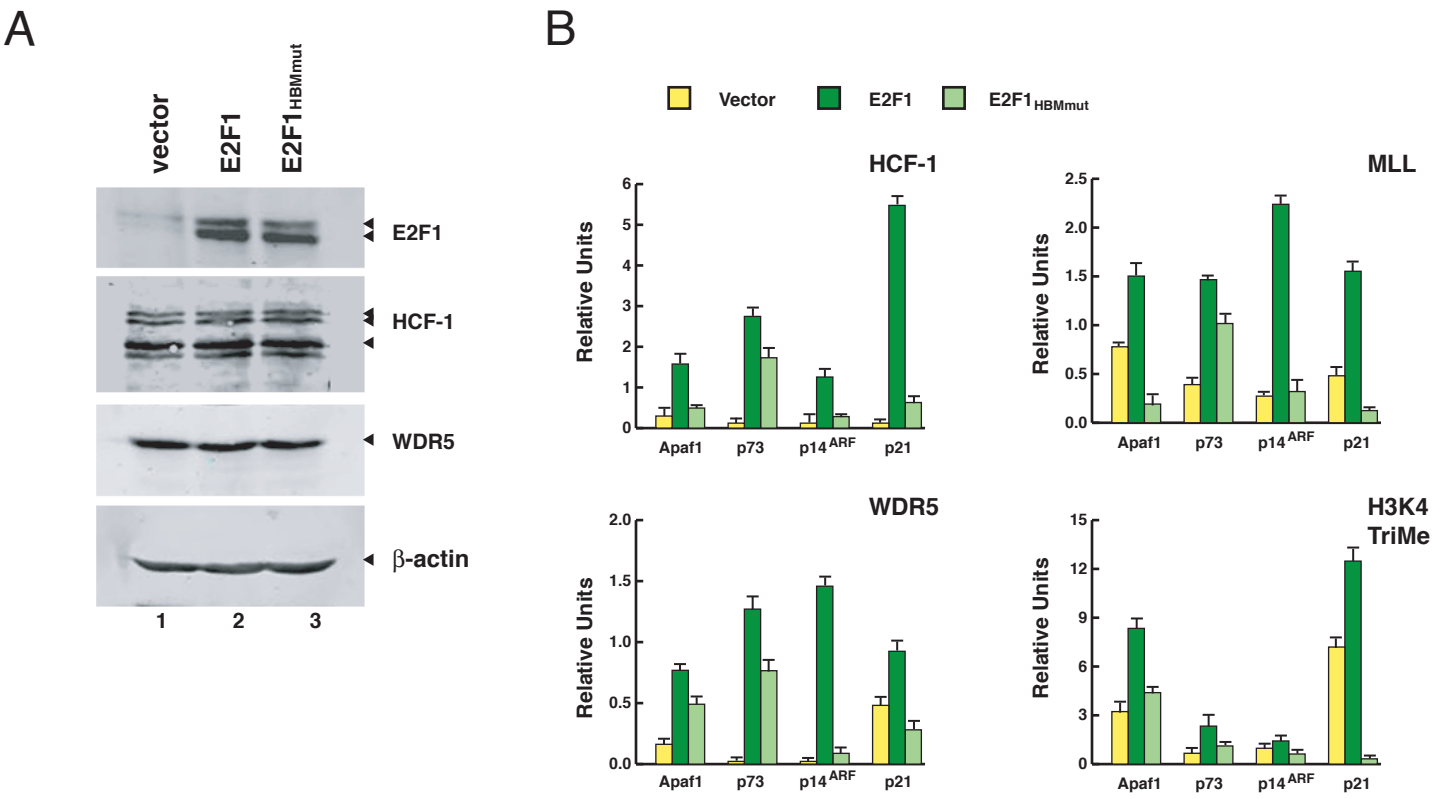
Reverse GTCTGGAGAAGAATCCAGG

Antibodies

Antisera used in these experiments were: polyclonal anti-HCF-1$_N$ subunit, anti-WDR5, anti-β actin, anti-E2F1 for immunoblots (KH95), anti-E2F1 for ChIP and immunoprecipitation (sc-193), anti-MLL$_C$ (clone 9-12) ; anti-trimethyl-Histone H3K4; ALEXA FLUOR 680 goat anti-rabbit and ALEXA FLUOR 680 goat anti-mouse have been described before (Tyagi et al., 2007); p53 (sc-6243, Santa Cruz biotechnology); anti-PCNA (Transduction Laboratories); ALEXA FLUOR 488 goat anti-rabbit, ALEXA FLUOR 546 goat anti-mouse (Invitrogen); anti 53BP1 (kind gift of T. Halazonetis, University of Geneva); anti RPA 70 and RPA 34 antibody (kind gift of B. Stillman, Cold Spring Harbor Laboratory)

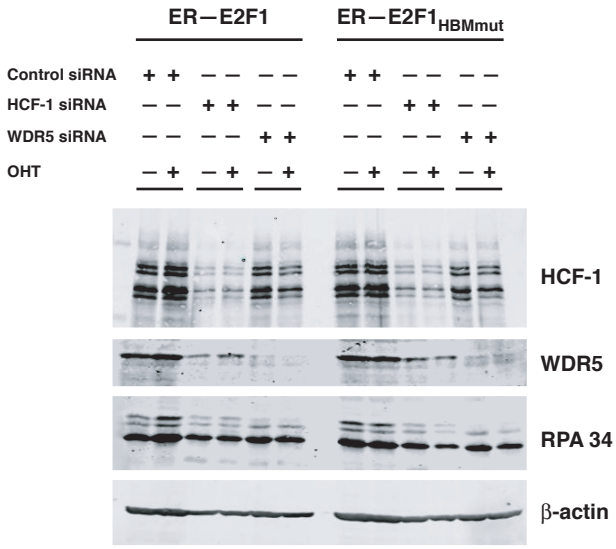


Supplementary Figure S1

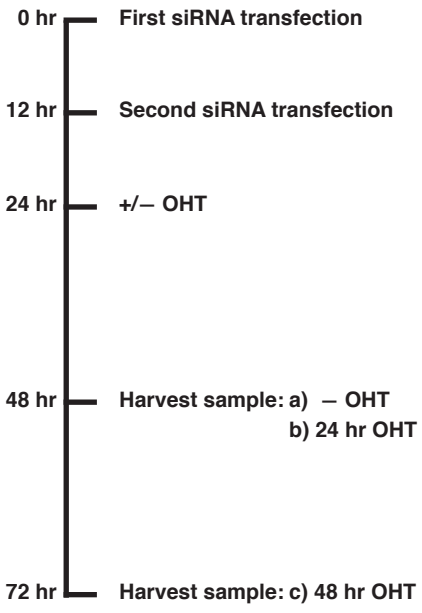


Supplementary Figure S2

A



B

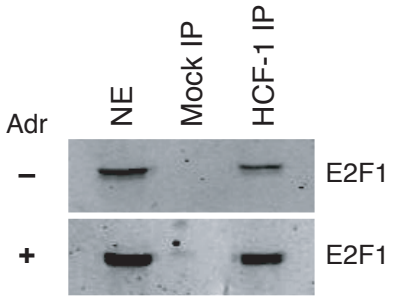


C

siRNA	OHT	hrs with OHT	Exp 1		Exp 2		Av of Exp 1 & 2	SD
			% sub-G1	Fold Inc	% sub-G1	Fold Inc		
Control	-	0	5.6	1	6.25	1	1	
Control	+	24	14.6	2.6	15.3	2.44	2.52	0.08
Control	+	48	19	3.39	22.8	3.64	3.51	0.12
HCF-1	-	0	12.9	1	15	1	1	
HCF-1	+	24	15.2	1.17	18.02	1.2	1.18	0.015
HCF-1	+	48	16.4	1.27	22.3	1.48	1.37	0.1
WDR5	-	0	16.6	1	17.9	1	1	
WDR5	+	24	17.2	1.03	21.7	1.21	1.12	0.09
WDR5	+	48	18.4	1.1	22	1.22	1.16	0.06

Supplementary Figure S3

A



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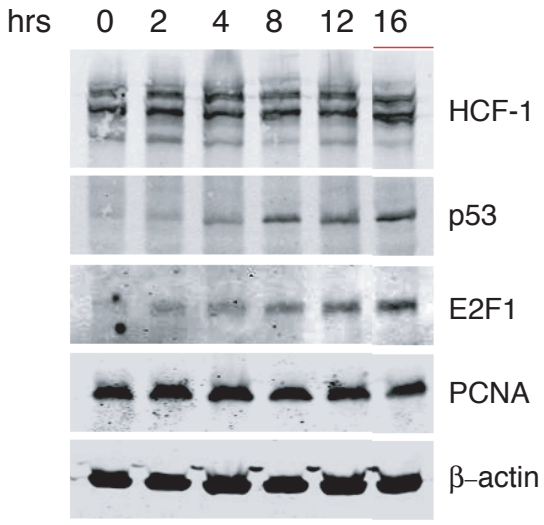

C

siRNA	Adr	hrs with Adr	Exp 1		Exp 2		Av of Exp 1 & 2	SD
			% sub-G1	Fold Inc	% sub-G1	Fold Inc		
Control	-	0	4.3	1	3.9	1	1	
Control	+	24	14.3	3.32	11.4	2.92	3.12	0.2
Control	+	48	23.8	5.53	24.6	6.31	5.92	0.38
HCF-1	-	0	11.5	1	11.9	1	1	
HCF-1	+	24	12.3	1.07	13.2	1.11	1.08	0.01
HCF-1	+	48	18.7	1.62	21.4	1.79	1.71	0.08
WDR5	-	0	10.3	1	9.3	1	1	
WDR5	+	24	11.9	1.15	10.3	1.11	1.13	0.02
WDR5	+	48	23	2.23	20.6	2.21	2.22	0.01

Supplementary Figure S4

Figure Legends

Figure **S1**

A Immunoblot analysis of U2OS cell extracts stably expressing empty vector, ER-E2F1, or ER-E2F1$_{HBMmut}$ are shown. Endogenous (E2F1) and recombinant (ER-E2F1) E2F1 proteins were visualized using anti-E2F1 antisera.

B Immunoblot analysis of control or E2F1 siRNA treated U2OS cell extracts stably expressing ER-E2F1 or ER-E2F1$_{HBMmut}$ are shown. (The difference in ER-E2F1 and ER-E2F1$_{HBMmut}$ mobility clearly seen in lanes 5 and 6 is a phosphatase-sensitive effect, which varies with electrophoresis conditions.)

Figure **S2**

A Immunoblot analysis of E2F1, HCF-1, WDR5, and β-actin proteins (as indicated) from U2OS cell extracts transfected with plasmid encoding empty vector, E2F1, or E2F1$_{HBMmut}$ mutant and harvested 24-hrs post-transfection, and used for the ChIP experiment in Fig. **5B** are shown.

B Quantitation of HCF-1, MLL, WDR5, and H3K4 trimethylation ChIP analyses of U2OS cells transfected with plasmids encoding empty vector, E2F1 or E2F1$_{HBMmut}$, by triplicate real-time PCR, of indicated promoters is shown. The cells samples are the same as in Fig. **5B** and are similarly normalized to E2F1 levels.

Figure **S3**.

A Immunoblot analysis of HCF-1, WDR5, RPA34, and β-actin proteins from U2OS cells stably expressing ER-E2F1 or ER-E2F1$_{HBMmut}$ and treated with control, HCF-1 or WDR5 siRNA for 72 hrs are shown. The cells were induced with OHT as indicated.

B Schematic outline of experiment performed in Fig. **6C**.

C Raw data (% sub-G1) and calculated relative-fold increase (Fold Inc) obtained in Fig **6C**.

Figure **S4**.

A Association of endogenous HCF-1 and E2F1, in nuclear extracts from HeLa cell treated with adriamycin (Adr) for 16 h. HCF-1 or mock immunoprecipitates (IP) of adriamycin treated (+) or untreated (–) samples were probed for E2F1. NE, input nuclear extract (3%).

B Immunoblot analysis of endogenous levels of HCF-1, p53, E2F1, PCNA and β-actin proteins from U2OS cells treated with 2μg/ml Adriamycin and harvested after indicated times are shown.

C Raw data (% sub-G1) and calculated relative-fold increase (Fold Inc) obtained in Fig **7C**.