Expanded View Figures

A

Protein	Phosphorylated peptide	Position	Fold change +Eto (1h)
E2F7*	KQKLARHGSFNT	410	108.3
	SPVMSRSHSVVQ	840	3.12
E2F8#	KPSFTRHPSLIK	395	>200
	ENDRRKISSAPS	412	>200
	RDCLHEHLSGDE	102	1.02

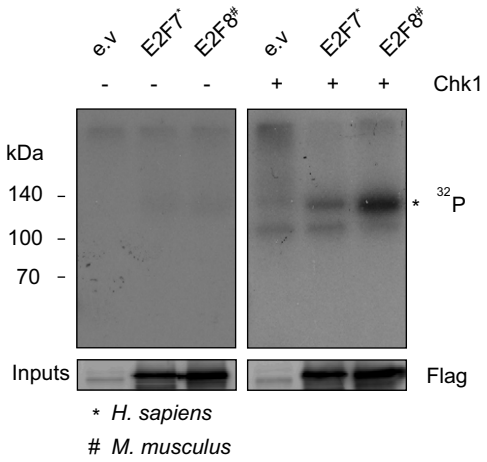
* *H. sapiens*
\# *M. musculus*

B

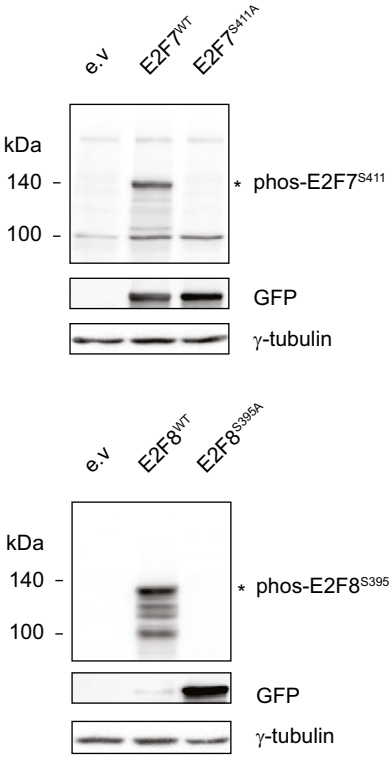
Protein	Phosphorylated peptide	Position	Fold change +Chk1
E2F7*	SPVMSRSHSVVQ	833	21.9
	KQKLARHGSFNT	410	8.34
	HSVVQQPESPVY	840	1.25
E2F8#	PHKRGLMKSPLH	20	2.89
	KPSFTRHPSLIK	395	1.13
	QRRATNHDSPVL	780	1.13

* *H. sapiens*
\# *M. musculus*

C



D



E

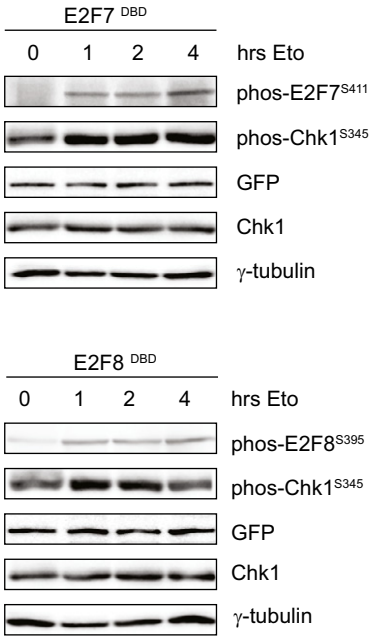


Figure EV1.

Figure EV1. Chk1 phosphorylates atypical E2Fs.

A Mass spectrometry data showing phosphorylation of E2F7 and E2F8 in response to 1-h etoposide-induced DNA damage. HEK cells were transfected with Flag-tagged E2F7 or E2F8 and immunoprecipitated with anti-Flag resins. Fold change indicates the phosphorylated peptide enrichment ratios in etoposide- versus DMSO-treated condition.

B Mass spectrometry data showing phosphorylation of precipitated E2F7 and E2F8 incubated with recombinant active Chk1. HEK cells were transfected with Flag-tagged E2F7/8, and cell lysates were precipitated with anti-Flag resins. Fold change indicates the intensity of phosphorylation with Chk1 versus without Chk1.

C Chk1 kinase assay for human E2F7 and mouse E2F8. HEK cells were transfected with indicated plasmids, and cell lysates were precipitated with anti-Flag resins. Precipitated E2F7 and E2F8 were incubated with or without recombinant active Chk1 and radiolabeled ^{32}P, and then, samples were loaded on a standard SDS–PAGE gel for exposure. Asterisk indicates the approximate expected molecular weight of Flag-tagged E2F7 and E2F8 (~115 and ~105 kDa, respectively).

D Immunoblots showing the detection of phosphorylated EGFP-tagged E2F7^{S411} and E2F8^{S395} using antibodies recognizing the Chk1 phosphorylation sites in E2F7 and E2F8. HEK cells were transfected with indicated plasmids for 48 h and treated with etoposide for 8 h prior to lysis to ensure high levels of active Chk1. Asterisks indicate the full-length fusion proteins (~140 kDa).

E Positive correlation between Chk1 activation and E2F7/8 phosphorylation. HEK cells were transfected with DNA-binding domain mutant versions of E2F7 and E2F8 (DBD) for 48 h. Cells were treated with etoposide and were harvested at the indicated time points after the treatment. Whole-cell lysates were subjected to immunoblotting.

Source data are available online for this figure.

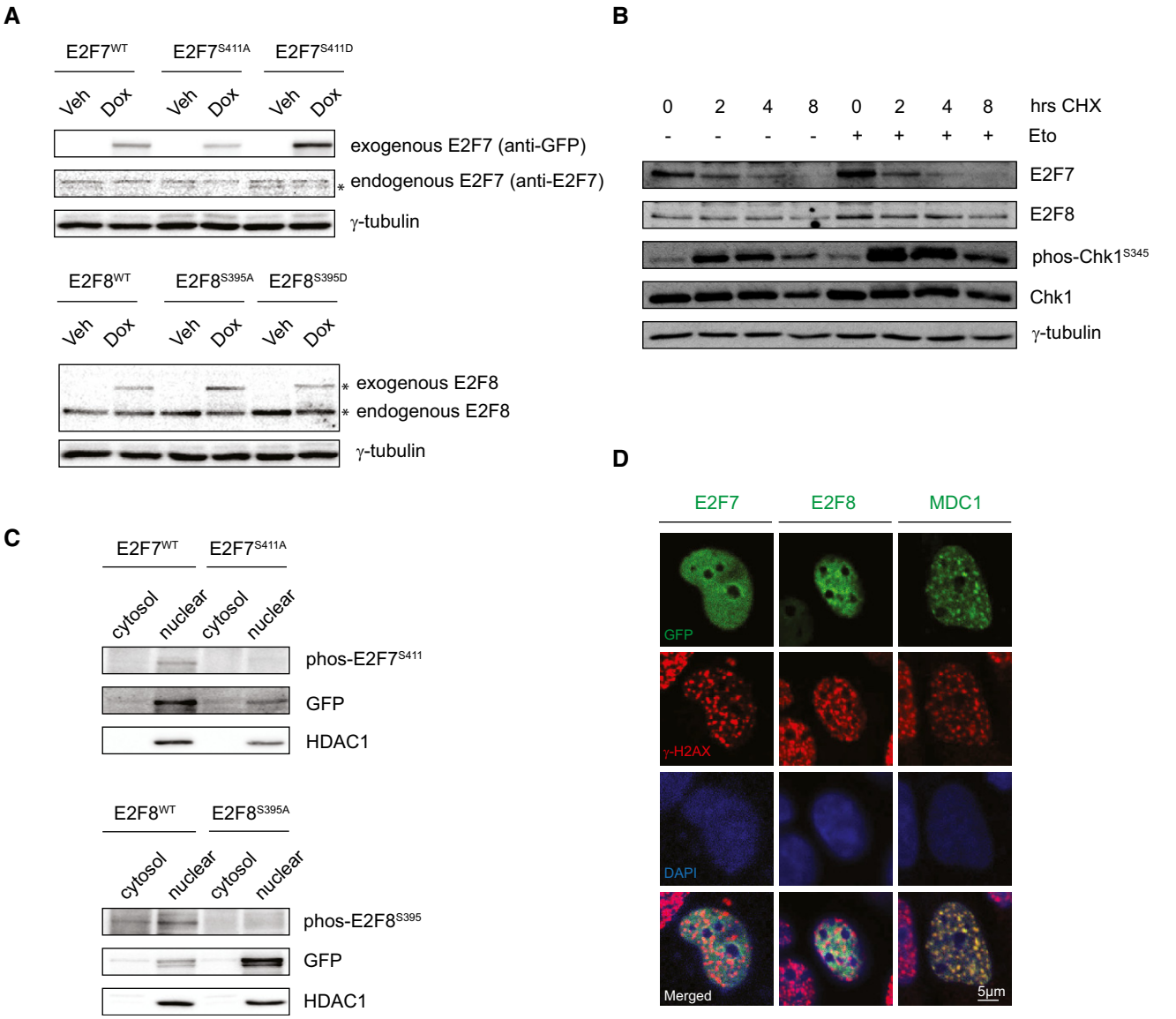


Figure EV2. Chk1-dependent phosphorylation does not cause stabilization or subcellular relocalization of atypical E2Fs.

A Protein expression of endogenous and exogenous atypical E2Fs in the HeLa/TO cell lines. EGFP-tagged wild-type, alanine and aspartic mouse constructs were incorporated in the HeLa/TO-inducible system by single colony selection. Cells were then treated either without (Veh) or with (Dox) doxycycline for 24 h. Exogenous E2F7 was measured by anti-GFP antibody, and endogenous E2F7 was measured by anti-human E2F7 antibody. For E2F8, both exogenous and endogenous forms were detected by a single E2F8 antibody. Asterisks indicate specific detections from the antibodies.

B Protein expression of endogenous E2F7, E2F8, phos-Chk1^{S345}, and total Chk1 in U2OS cells treated with cycloheximide (CHX), in the presence or absence of etoposide.

C E2F7 and E2F8 proteins are localized in the nucleus. Inducible cell lines were treated with doxycycline and etoposide for 16 h, followed by isolation of cytosolic and nuclear protein fractions. HDAC1 expression was used as an indicator for enrichment of nuclear proteins.

D Immunofluorescence showing the localization of E2F7, E2F8, and MDC1 in response to camptothecin treatment for 8 h in U2OS cells. E2F7, E2F8, and MDC1 were labeled with GFP, and DNA damage sites are detected with an antibody against γ-H2AX. DNA was stained with DAPI. Scale bar: 5 μm.

Source data are available online for this figure.

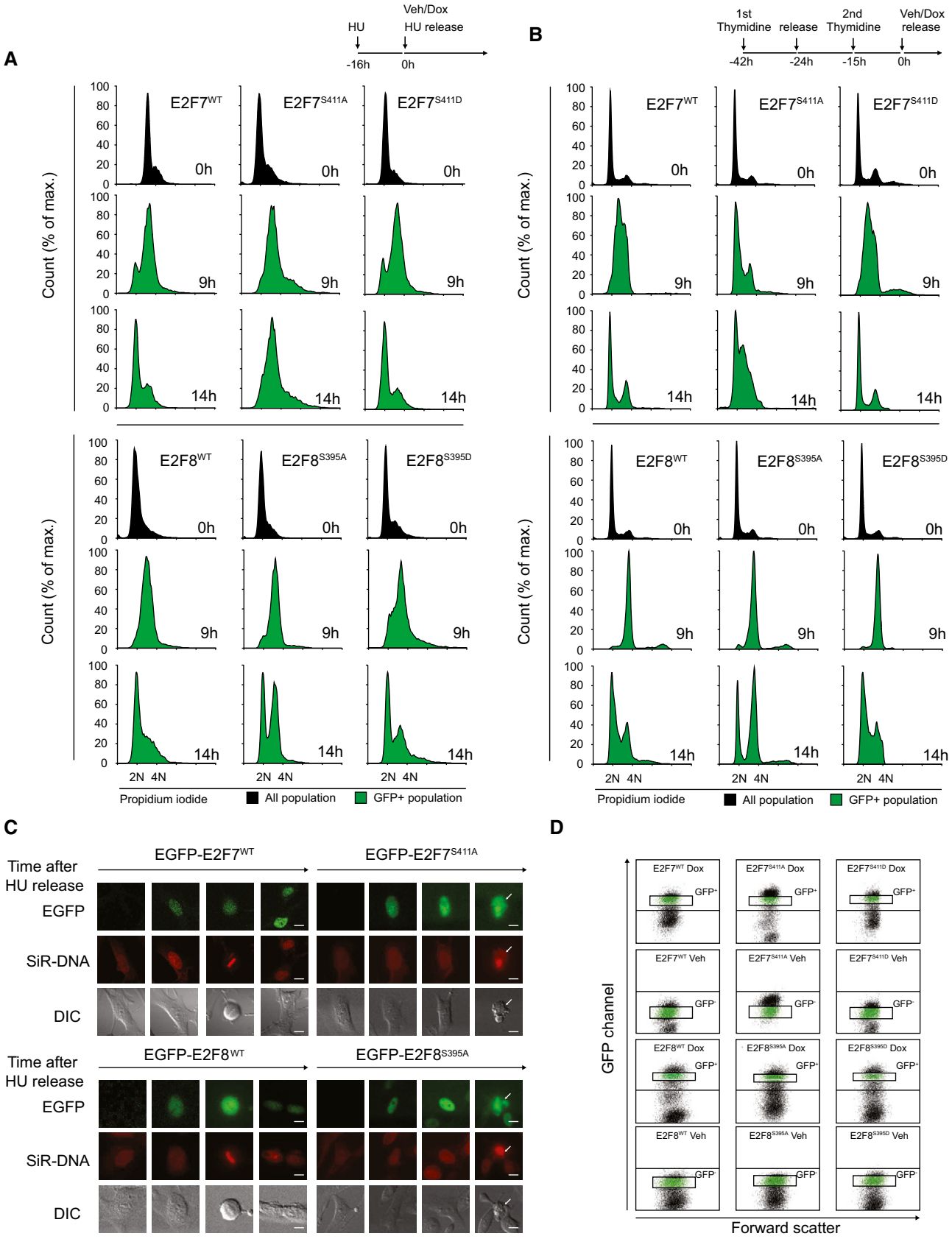

A
HU
Veh/Dox
HU release
-16h
0h
E2F7WT
E2F7S411A
E2F7S411D
E2F8WT
E2F8S395A
E2F8S395D
0h
9h
14h
Count (% of max.)
2N 4N
Propidium iodide
All population
GFP+ population
B
1st Thymidine
release
2nd Thymidine
Veh/Dox release
-42h
-24h
-15h
0h
C
Time after HU release
EGFP-E2F7WT
EGFP-E2F7S411A
EGFP-E2F8WT
EGFP-E2F8S395A
EGFP
SiR-DNA
DIC
D
E2F7WT Dox
E2F7S411A Dox
E2F7S411D Dox
E2F8WT Dox
E2F8S395A Dox
E2F8S395D Dox
E2F7WT Veh
E2F7S411A Veh
E2F7S411D Veh
E2F8WT Veh
E2F8S395A Veh
E2F8S395D Veh
GFP+
GFP-
GFP channel
Forward scatter

Figure EV3.

Figure EV3. Prevention of Chk1-mediated phosphorylation of atypical E2Fs delays cell cycle progression and reduces cell survival.

A Cell cycle profile analysis by flow cytometry confirmed that over-expression of non-phosphorylatable forms of E2F7 or E2F8 delays cell cycle progression after HU release. HeLa/TO cells expressing wild-type or mutant forms of E2F7/8 were harvested at 0, 9, and 14 h after HU release and doxycycline induction.

B Cell cycle profile analysis by flow cytometry confirmed that over-expression of non-phosphorylatable forms of E2F7 or E2F8 delays cell cycle progression after double thymidine block/release. HeLa/TO cells expressing wild-type or mutant forms of E2F7/8 were harvested at 0, 9, and 14 h after double thymidine block/release and doxycycline induction.

C Representative montage figures from the live cell imaging show that over-expression of alanine forms of E2F7/8 induces cell blebbing (arrows), interpreted as cell death. Scale bar: 10 μm.

D FACS sorting of cells expressing wild-type and mutant E2F7 and E2F8. Sorting gates (rectangles) were set to isolate cells with comparable levels of expression from all E2F7/8-EGFP-expressing (Dox) cell lines.

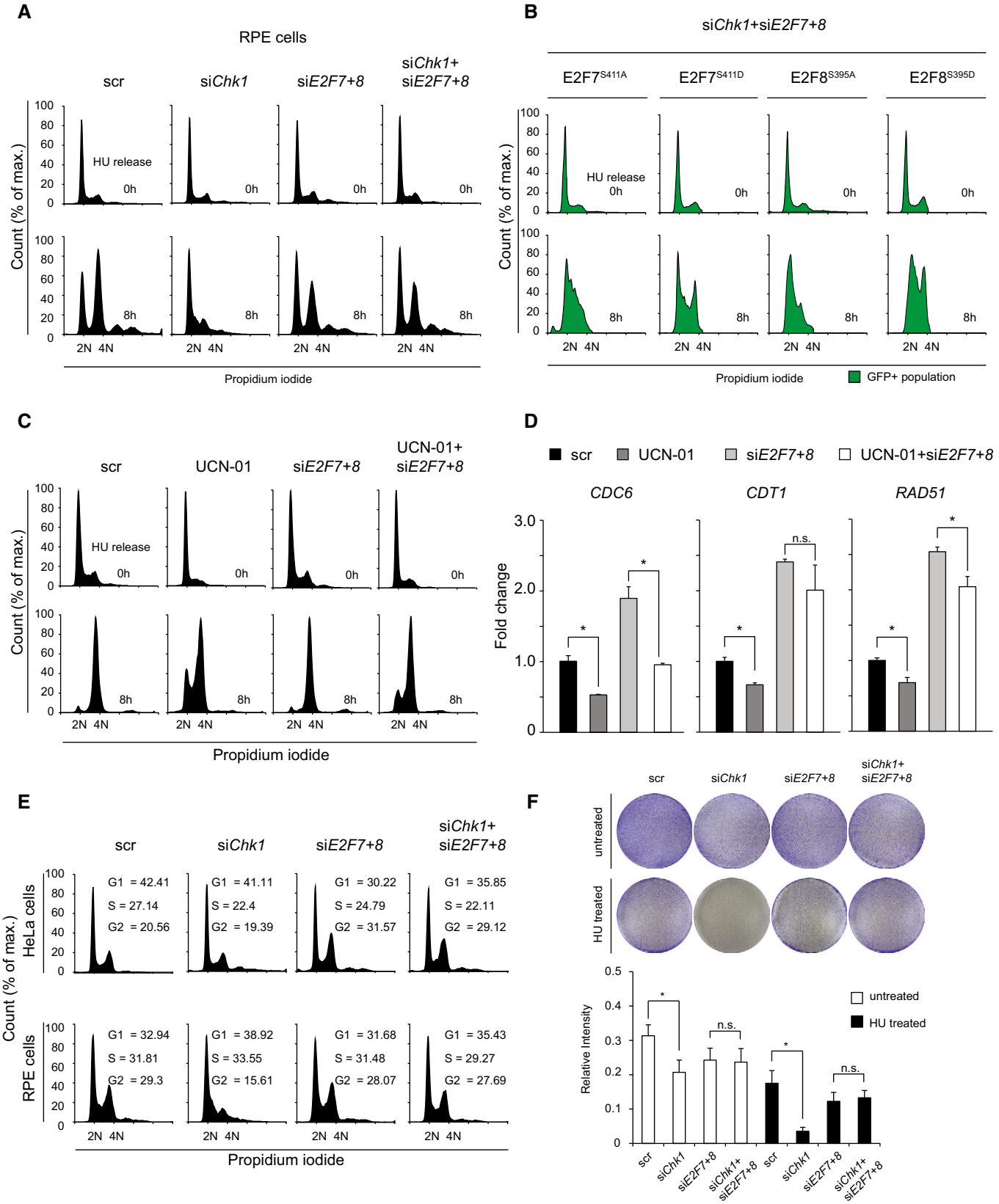

A
RPE cells
scr
siChk1
siE2F7+8
siChk1+ siE2F7+8
Count (% of max.)
HU release
0h
8h
2N 4N
Propidium iodide
B
siChk1+siE2F7+8
E2F7S411A
E2F7S411D
E2F8S395A
E2F8S395D
GFP+ population
C
UCN-01
UCN-01+ siE2F7+8
D
scr
UCN-01
siE2F7+8
UCN-01+siE2F7+8
CDC6
CDT1
RAD51
Fold change
n.s.
E
HeLa cells
RPE cells
G1 = 42.41
S = 27.14
G2 = 20.56
G1 = 41.11
S = 22.4
G2 = 19.39
G1 = 30.22
S = 24.79
G2 = 31.57
G1 = 35.85
S = 22.11
G2 = 29.12
G1 = 32.94
S = 31.81
G2 = 29.3
G1 = 38.92
S = 33.55
G2 = 15.61
G1 = 31.68
S = 31.48
G2 = 28.07
G1 = 35.43
S = 29.27
G2 = 27.69
F
untreated
HU treated
Relative Intensity

Figure EV4.

Figure EV4. Chk1 inhibition induces an E2F7/8-dependent cell cycle arrest and reduces cell survival.

A FACS cell cycle profiles at 0 and 8 h after HU release from propidium iodide-stained RPE cells incubated with siRNAs directed against *Chk1* and *E2F7/8*. Scrambled (scr) siRNA was used as control.

B FACS cell cycle profiles at 0 and 8 h after HU release from propidium iodide-stained GFP-positive HeLa/TO cells transfected with siRNA against *Chk1* and *E2F7/8*. Doxycycline was added to the cells 16 h before HU release.

C FACS cell cycle profiles from propidium iodide-stained HeLa cells treated with UCN-01. HeLa cells were first transfected with scramble or si*E2F7+8*, then exposed to HU and the Chk1 inhibitor UCN-01 for 16 h, and eventually harvested at 0 and 8 h after HU release.

D Transcript levels of E2F7/8 target genes after 16 h of HU treatment, measured by qPCR. Fold changes were adjusted to the scrambled condition.

E Cell cycle distribution of HeLa and RPE cells transfected with siRNA against *Chk1* and *E2F7/8* under unperturbed condition. Scrambled (scr) siRNA was used as control.

F Clonogenic survival assay of HeLa cells transfection with siRNA against *Chk1* and *E2F7/8* under un-treated or HU-treated condition. Cells were transfected with indicated siRNA for 24 h and reseeded at a low density and grow for 4 days. Scrambled (scr) siRNA was used as control. Histogram shows the quantification of relative intensity from each condition (n = 2 independent experiments).

Data information: In (D and F), data represent mean ± SEM (n = 3); *P < 0.05 (Student's t-test) or n.s. for not significant.

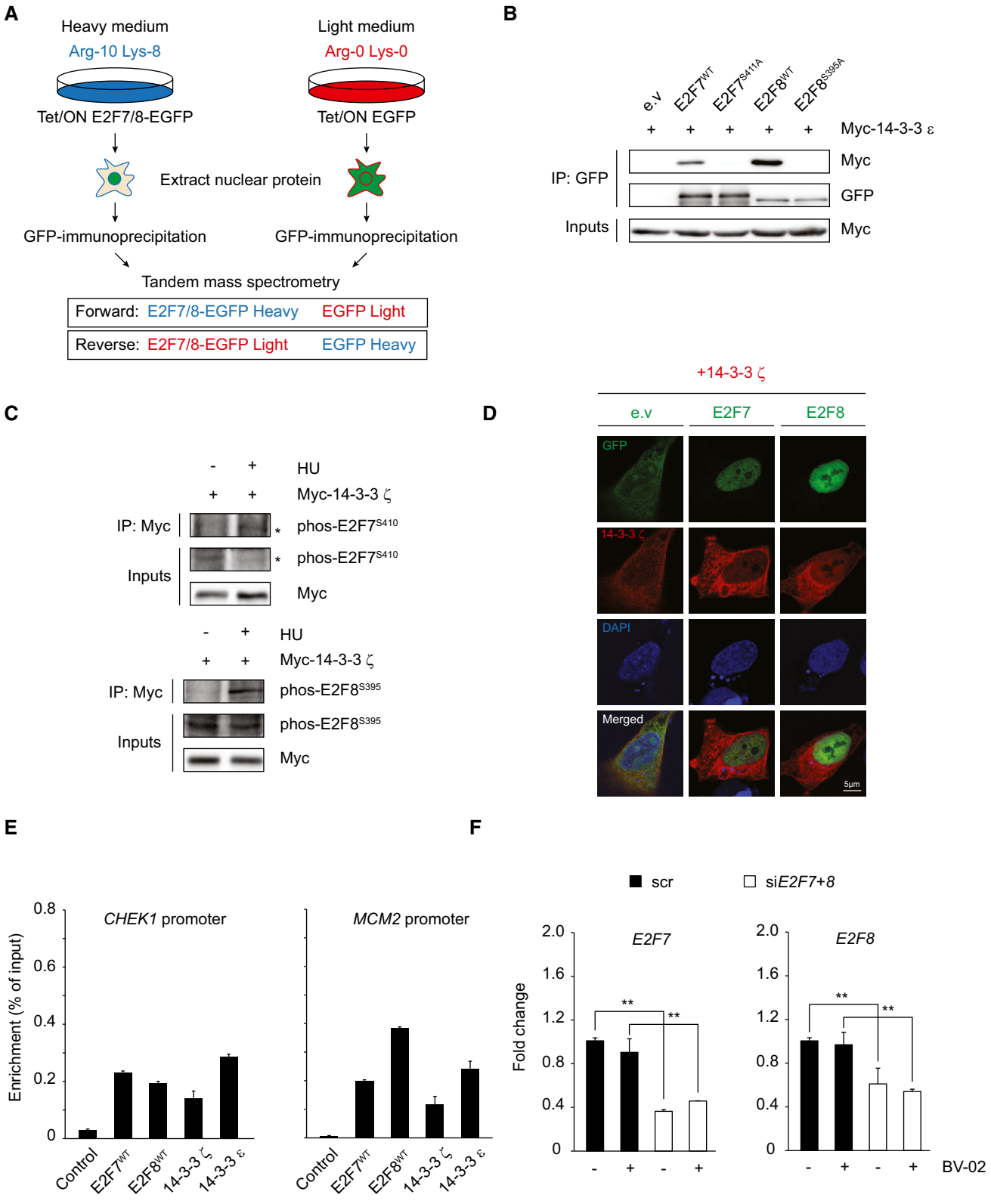

A
Heavy medium
Arg-10 Lys-8
Light medium
Arg-0 Lys-0
Tet/ON E2F7/8-EGFP
Tet/ON EGFP
Extract nuclear protein
GFP-immunoprecipitation
GFP-immunoprecipitation
Tandem mass spectrometry
Forward: E2F7/8-EGFP Heavy EGFP Light
Reverse: E2F7/8-EGFP Light EGFP Heavy
B
e.v
E2F7WT
E2F7S411A
E2F8WT
E2F8S395A
Myc-14-3-3 ε
IP: GFP
Myc
GFP
Inputs
Myc
C
HU
Myc-14-3-3 ζ
IP: Myc
phos-E2F7S410
Inputs
phos-E2F7S410
Myc
HU
Myc-14-3-3 ζ
IP: Myc
phos-E2F8S395
Inputs
phos-E2F8S395
Myc
D
+14-3-3 ζ
e.v
E2F7
E2F8
GFP
14-3-3 ζ
DAPI
Merged
5μm
E
CHEK1 promoter
MCM2 promoter
Enrichment (% of input)
Control
E2F7WT
E2F8WT
14-3-3 ζ
14-3-3 ε
F
scr
siE2F7+8
E2F7
E2F8
Fold change
BV-02

Figure EV5.

Figure EV5. 14-3-3 proteins interact with E2F7/8 at the promoter regions.

A Experimental procedures of the SILAC assay to identify binding partners of E2F7 and E2F8.

B 14-3-3 ε interacts with wild-type but not alanine mutant E2F7 and E2F8 *in vitro*. HEK 293T cells were transfected as indicated, and lysates were precipitated with GFP-Trap beads and immunoblotted with antibodies directed against GFP or Myc. Inputs confirm similar expression of Myc-14-3-3 ε in all samples.

C 14-3-3 ζ interacts with endogenous phosphorylated E2F7 and E2F8 *in vivo*. HEK 293T cells were transfected with Myc-14-3-3 ζ and treated with or without HU. Lysates were precipitated with anti-Myc antibodies and immunoblotted with antibodies directed against the Chk1 phosphorylation site of E2F7^{S410} or E2F8^{S396} (asterisks indicate the signal). Inputs confirm similar expression of Myc-14-3-3 ζ in all samples.

D 14-3-3 ζ does not alter the nuclear localization of E2F7/8. U2OS cells were transfected with Myc-14-3-3 ζ with either empty vector, EGFP wild-type E2F7 or E2F8 followed by immunofluorescence staining. DAPI was used to stain nuclear DNA. Scale bar: 5 μm.

E Chromatin immunoprecipitation (ChIP) demonstrated that 14-3-3 proteins bind to the E2F7/8 target gene promoters. HEK cells were transfected with either PEI reagent alone (control) or indicated plasmids. 48 h after transfection, cells were harvested for ChIP assay and followed by qPCR. Histogram represents the enrichment ratio (bound/input) in *E2Fs* target gene promoters. A primer set designed against a distal region in the *E2F1* gene served as a negative control.

F Validation of E2F7 and E2F8 knockdown efficiency of experiments shown in Fig 5F. *E2F7* and *E2F8* mRNA levels was determined by qPCR.

Data information: In (D, F), data represent mean ± SEM ($n = 3$); $^{**}P < 0.01$ (Student's *t*-test).
Source data are available online for this figure.