SUPPLEMENTAL INFORMATION

Figure Legends

Figure S1 related to Figure 1. MLN4924 differentially regulates UBE2M and UBE2F.

(**A&B**) H358 cells were treated with MLN4924 at indicated concentrations (24 h) or time points, followed by IB using indicated Abs.

(**C**) H358 cells were treated with different doses of MLN4924 for 24 h, followed by qRT-PCR. Data are represented as mean ± SEM of three independent experiments. *** $p < 0.001$, based on One-way ANOVA.

(**D**) H358 cells were treated with MLN4924 (0.3 μM) for 24 h and then switched to fresh medium (10% FBS without MLN4924) containing cycloheximide (CHX) and incubated for indicated time periods before being harvested for IB.

(**E**) Measurement of UBE2M and UBE2F T1/2: the band density was quantified using ImageJ software and plotted.

(**F&G**) H358 cells were transfected with indicated siRNAs and treated with MLN4924 for 24 h, followed by IB (F) or qRT-PCR assay (G). Data are represented as mean ± SEM of three independent experiments. *** $p < 0.001$, based on One-way ANOVA.

Figure S2 related to Figure 2. UBE2M is transcriptionally activated by HIF-1α and AP1.

(A) Bar graph presentation of the location of AP1 binding sites and HIF-1α site in the promoter or intron-1of human *UBE2M* gene.

(**B-E**) SK-MES-1 or A427 cells were treated with different doses of $CoCl_2$ (B&D) or TPA (C&E) for 24 h, followed by IB with indicated Abs (B&C) or qRT-PCR (D&E). Data are represented as mean ± SEM of three independent experiments**.** * $p < 0.05$, *** $p < 0.001$, based on One-way ANOVA (D&E).

(**F**) ChIP analysis: H1299 cells were treated with indicated compounds for 24 h. Cellular DNA was isolated and used directly as PCR templates (input), or subjected to immunoprecipitation in the absence (Ab-) or presence of IgG or Ab against c-JUN.

The DNA precipitates were subjected to PCR amplification using primer sets flanking the AP1 binding site-1.

Figure S3 related to Figure 3. UBE2M negatively regulates UBE2F protein levels.

(**A**) H358 cells were firstly transfected with si-Cont or si-UBE2M-2 and then treated with indicated reagents. Cells were harvested for IB with indicated Abs.

(**B-E**) H358 (B) or A427 (C-E) cells were transfected with indicated plasmids, followed by IB with indicated Abs, or qRT-PCR (bottom panels, B&C), or were switched to fresh medium (10% FBS) containing cycloheximide (CHX) and incubated for indicated time periods, followed by IB and quantification of band density (E).

(**F-I**) A427 or H358 cells were transfected with two independent siRNAs targeting UBE2M or si-Cont for 48 h, followed by IB with indicated Abs (F) or qRT-PCR (**G&H**), or were switched to fresh medium (10% FBS) containing cycloheximide (CHX) and incubated for indicated time periods, followed by IB and quantification of band density (I).

Figure S4 related to Figure 4. CUL3 negatively regulates UBE2F protein levels.

(**A&B**) A427 cells were transfected with siRNAs oligoes targeting different cullins (A) or indicated cullin expressing plasmids (B), followed by IB with indicated Abs.

(**C-F**) H358 cells were transfected with siRNA oligo targeting CUL3 (C&E) or plasmid expressing CUL3 (D&F), followed by IB or qRT-PCR, or switched to fresh medium (10% FBS) containing cycloheximide (CHX) post-transfection and incubated for indicated time periods before being harvested for IB (top panels, E&F). The band density was quantified (bottom panels, E&F).

(**G-I**) Various cell lines were transfected with indicated plasmid or siRNA oligoes, followed by HA-IP and HA/Myc IB (G); or followed by direct IB with indicated Abs (H&I).

Figure S5 related to Figure 5. Keap1 regulates UBE2F protein levels.

(**A**) Evolutionary conservation of Keap1 binding motif on UBE2F among different

species.

(**B-H**) A427 or H358 cells were transfected with indicated plasmids or siRNA oligoes, followed by qRT-PCR (B, D, E, G) or IB (C&F) or switched to fresh medium (10% FBS) containing cycloheximide (CHX) post-transfection and incubated for indicated time periods before being harvested for IB (H).

(I) Mouse embryonic fibroblasts with *Keap1*$^{+/+}$ vs. *Keap1*$^{-/-}$ genotype were subjected to IB with indicated Abs.

(J) H358 cells were transfected with Myc-CUL3, followed by IP with anti-Myc Ab and IB with indicated Abs.

(K) Computer modeling of Keap1-Nrf2 binding. Three cancer-derived Keap1 mutants used in this study were marked.

(**L&M**) H358 or A427 cells were transfected with indicated plasmids or siRNA oligoes, followed by IB with indicated Abs.

Figure S6 related to Figure 6. DJ-1/Parkin is the major E3 for UBE2F ubiquitylation.

(**A**) H1299 cells were transfected with indicated plasmids alone or in combination with siRNA oligoes (Con, Control, C3, CUL3, K1, Keap1), followed by Ni-bead purification and IB with anti-UBE2F Ab (top). The whole cell extracts were subjected to direct IB with indicated Abs.

(**B&C**) H1299 cells were transfected with indicated plasmids, followed by IP with anti-Myc (B) or anti-HA (C), and IB with indicated Abs. Whole cell extracts (WCL) were subjected to direct IB with indicated Abs.

(**D**) H358 cells were treated with indicated compounds for 24 h, followed by IP with UBE2M Ab and IB with indicated Abs.

(E and F) H358 cells were transfected with indicated siRNAs oligoes, followed by IB with indicated Abs (E), or subjected to half-life study with cycloheximide (CHX) (F). The band density was quantified (bottom panel, F).

(**G&H**) H358 cells were transfected with indicated plasmids, followed by IB with indicated Abs (G), or subjected to protein half-life study with cycloheximide (H). The

band density was quantified (bottom panel, H).

(**I**) The 293 cells were individually transfected with Myc-DJ-1, FLAG-Parkin (FL-Parkin), FLAG-Keap1 (FL-Keap1), or Myc-CUL3, followed by IP purification with Tag Abs to serve as different source of E3. These E3s were added into a reaction mixture containing ATP, ubiquitin, E1, E2 (UBCH5C), and substrate (purified UBE2F protein), followed by *in vitro* ubiquitylation assay and IB with anti-UBE2F Ab.

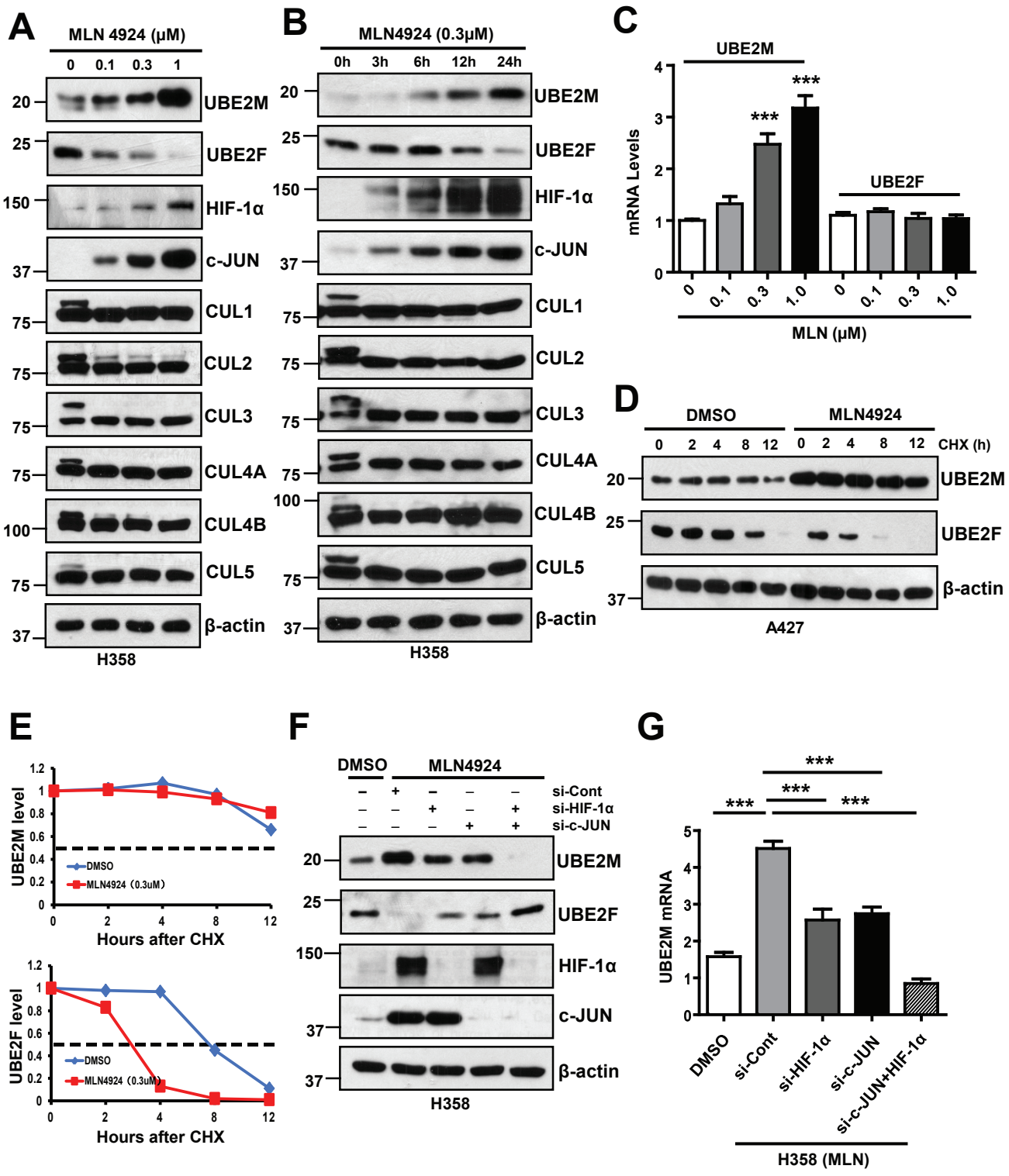
(**J**) 293 cells were individually transfected with FLAG-Parkin (FL-Parkin) or Myc-CUL3, and then pulled down with Tag Abs to serve as different source of E3 and incubated with E1, E2 (UBCH5C or UBE2M), and substrate (purified UBE2F protein), followed by *in vitro* ubiquitylation assay and IB with anti-UBE2F Ab.

Figure S7 related to Figure 7. UBE2M or Parkin is a tumor suppressor against lung cancer.

(**A-C**) Nude mice tumor tissues derived from H1299 cells expressing indicated plasmids were subjected to IB (A) or immunohistochemical staining of tumor sections with indicated Abs (B; Scale bar, 100 μm) with quantification of staining intensity (C). Data are represented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, based on One-way ANOVA (C).

(**D-F**) H358 cells were transfected with indicated plasmids or Lenti-virus targeting NOXA alone or in combination, followed by IB with indicated Ab (D), ATPlite assay for proliferation (E), and clonogenic assay for survival (F). Data are represented as mean ± SD of three independent experiments. ** $p < 0.01$, *** $p < 0.001$, based on Two-way ANOVA (E) or One-way ANOVA (F).

(**G**) Correlation of the levels among UBE2F, UBE2M, and Parkin proteins in lung cancer cells. Data were analyzed using SPSS software to obtain coefficient ($p < 0.001$, Pearson's test)


A
MLN 4924 (μM)
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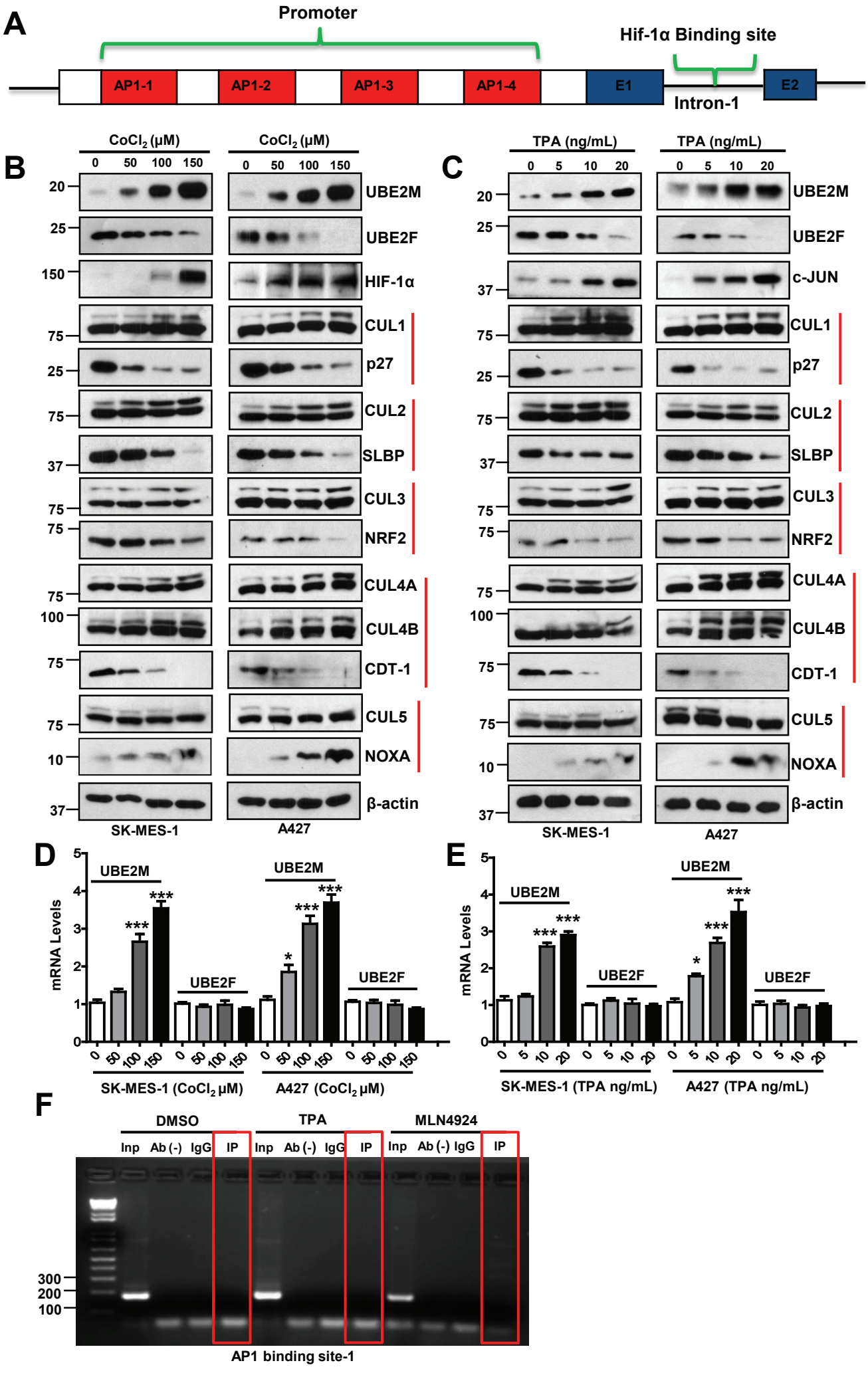
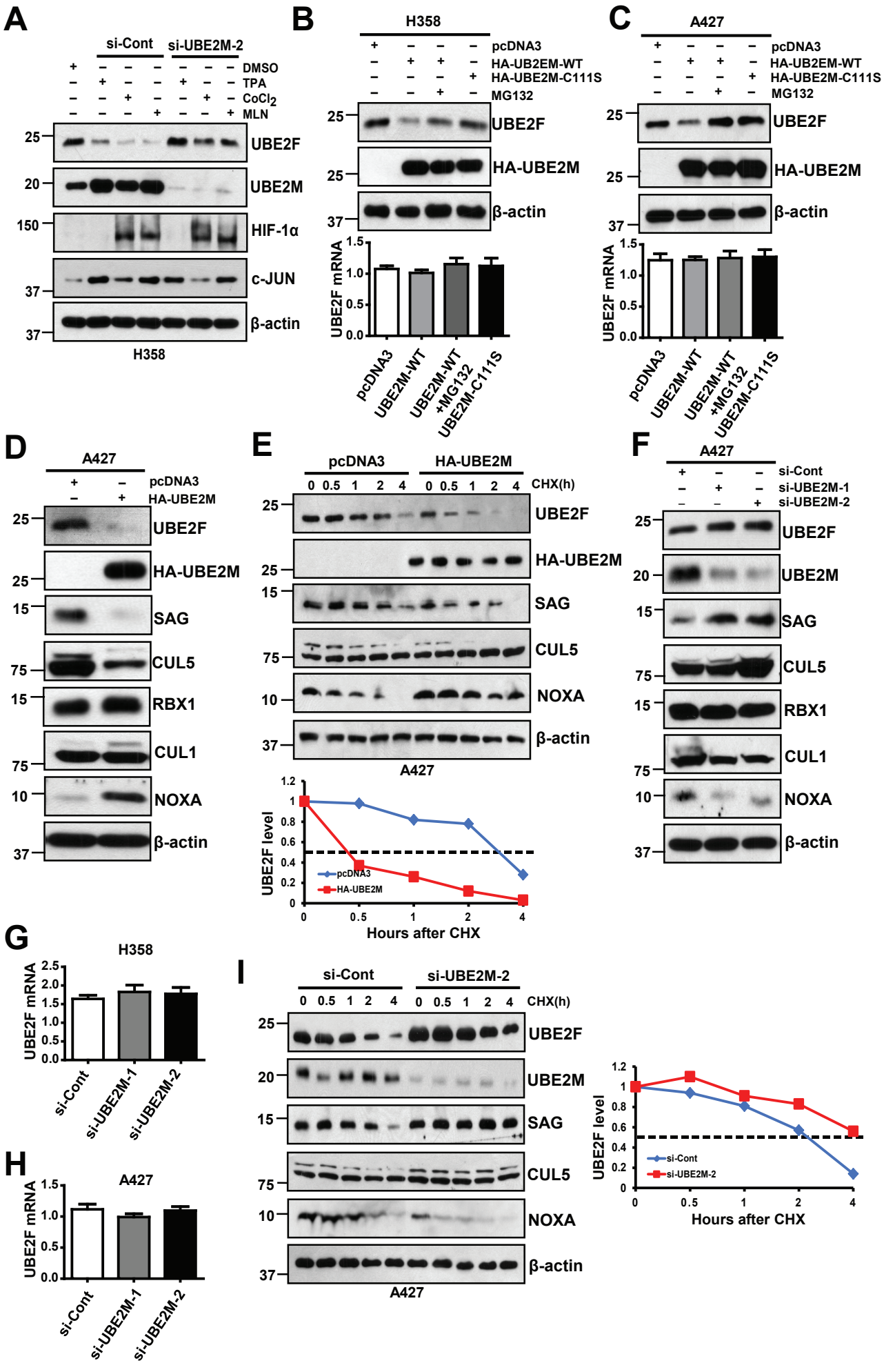
UBE2M
UBE2F
HIF-1α
c-JUN
CUL1
CUL2
CUL3
CUL4A
CUL4B
CUL5
β-actin
H358
B
MLN4924 (0.3μM)
0h 3h 6h 12h 24h
UBE2M
UBE2F
HIF-1α
c-JUN
CUL1
CUL2
CUL3
CUL4A
CUL4B
CUL5
β-actin
H358
C
UBE2M
UBE2F
mRNA Levels
***
MLN (μM)
D
DMSO
MLN4924
0 2 4 8 12 0 2 4 8 12 CHX (h)
UBE2M
UBE2F
β-actin
A427
E
UBE2M level
DMSO
MLN4924 (0.3uM)
Hours after CHX
UBE2F level
Hours after CHX
F
DMSO
MLN4924
si-Cont
si-HIF-1α
si-c-JUN
UBE2M
UBE2F
HIF-1α
c-JUN
β-actin
H358
G
UBE2M mRNA
***
DMSO
si-Cont
si-HIF-1α
si-c-JUN
si-c-JUN+HIF-1α
H358 (MLN)

Zhou et al., Figure S2

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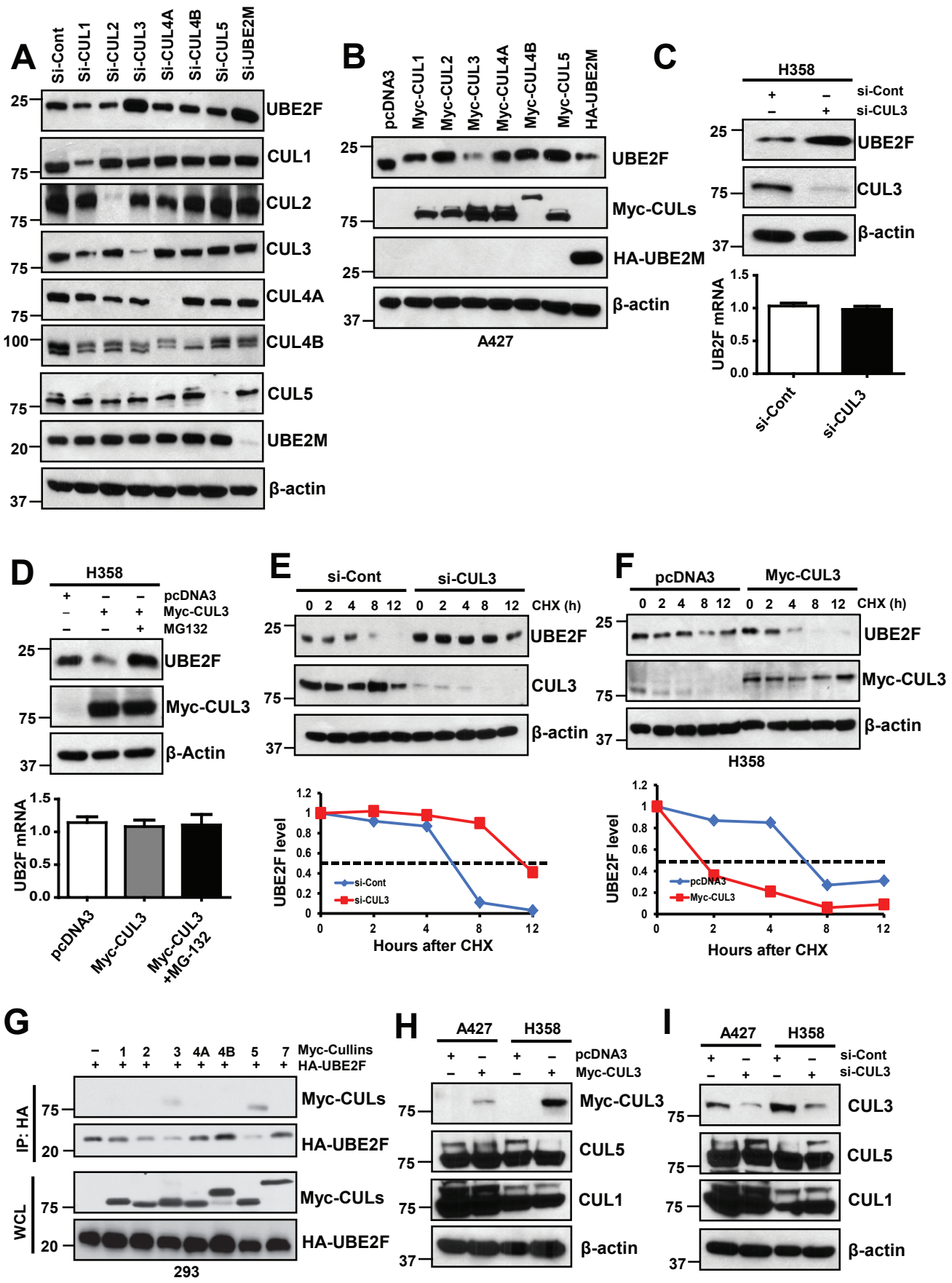
H358
B
H358
pcDNA3
HA-UB2EM-WT
HA-UBE2M-C111S
MG132
UBE2F
HA-UBE2M
β-actin
UBE2F mRNA
pcDNA3
UBE2M-WT
UBE2M-WT +MG132
UBE2M-C111S
C
A427
pcDNA3
HA-UB2EM-WT
HA-UBE2M-C111S
MG132
UBE2F
HA-UBE2M
β-actin
UBE2F mRNA
pcDNA3
UBE2M-WT
UBE2M-WT +MG132
UBE2M-C111S
D
A427
pcDNA3
HA-UBE2M
UBE2F
HA-UBE2M
SAG
CUL5
RBX1
CUL1
NOXA
β-actin
E
pcDNA3
HA-UBE2M
0 0.5 1 2 4 0 0.5 1 2 4 CHX(h)
UBE2F
HA-UBE2M
SAG
CUL5
NOXA
β-actin
A427
UBE2F level
pcDNA3
HA-UBE2M
Hours after CHX
F
A427
si-Cont
si-UBE2M-1
si-UBE2M-2
UBE2F
UBE2M
SAG
CUL5
RBX1
CUL1
NOXA
β-actin
G
H358
UBE2F mRNA
si-Cont
si-UBE2M-1
si-UBE2M-2
H
A427
UBE2F mRNA
si-Cont
si-UBE2M-1
si-UBE2M-2
I
si-Cont
si-UBE2M-2
0 0.5 1 2 4 0 0.5 1 2 4 CHX(h)
UBE2F
UBE2M
SAG
CUL5
NOXA
β-actin
A427
UBE2F level
si-Cont
si-UBE2M-2
Hours after CHX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Si-Cont
Si-CUL1
Si-CUL2
Si-CUL3
Si-CUL4A
Si-CUL4B
Si-CUL5
Si-UBE2M
UBE2F
CUL1
CUL2
CUL3
CUL4A
CUL4B
CUL5
UBE2M
β-actin
B
pcDNA3
Myc-CUL1
Myc-CUL2
Myc-CUL3
Myc-CUL4A
Myc-CUL4B
Myc-CUL5
HA-UBE2M
UBE2F
Myc-CULs
HA-UBE2M
β-actin
A427
C
H358
si-Cont
si-CUL3
UBE2F
CUL3
β-actin
UB2F mRNA
si-Cont
si-CUL3
D
H358
pcDNA3
Myc-CUL3
MG132
UBE2F
Myc-CUL3
β-Actin
UB2F mRNA
pcDNA3
Myc-CUL3
Myc-CUL3 +MG-132
E
si-Cont
si-CUL3
CHX (h)
UBE2F
CUL3
β-actin
UBE2F level
Hours after CHX
si-Cont
si-CUL3
F
pcDNA3
Myc-CUL3
CHX (h)
UBE2F
Myc-CUL3
β-actin
H358
UBE2F level
Hours after CHX
pcDNA3
Myc-CUL3
G
Myc-Cullins
HA-UBE2F
IP: HA
WCL
Myc-CULs
HA-UBE2F
293
H
A427
H358
pcDNA3
Myc-CUL3
Myc-CUL3
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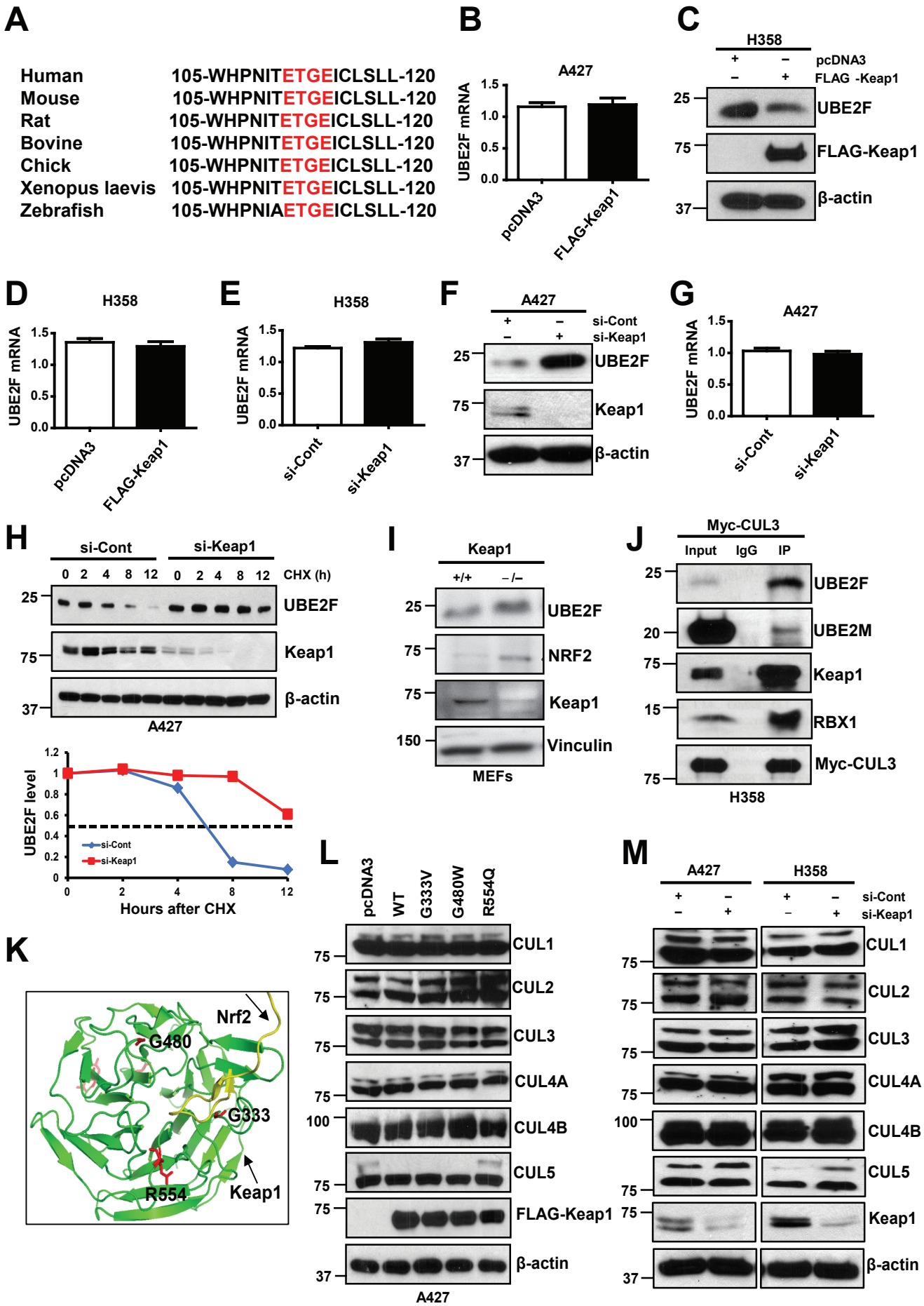
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Rat 105-WHPNITETGEICLSLL-120
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B
A427
UBE2F mRNA
pcDNA3
FLAG-Keap1
C
H358
pcDNA3
FLAG -Keap1
UBE2F
FLAG-Keap1
β-actin
D
H358
UBE2F mRNA
pcDNA3
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E
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F
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Keap1
β-actin
G
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si-Keap1
H
si-Cont
si-Keap1
CHX (h)
UBE2F
Keap1
β-actin
A427
UBE2F level
si-Cont
si-Keap1
Hours after CHX
I
Keap1
UBE2F
NRF2
Keap1
Vinculin
MEFs
J
Myc-CUL3
Input
IgG
IP
UBE2F
UBE2M
Keap1
RBX1
Myc-CUL3
H358
K
Nrf2
G480
G333
R554
Keap1
L
pcDNA3
WT
G333V
G480W
R554Q
CUL1
CUL2
CUL3
CUL4A
CUL4B
CUL5
FLAG-Keap1
β-actin
A427
M
A427
H358
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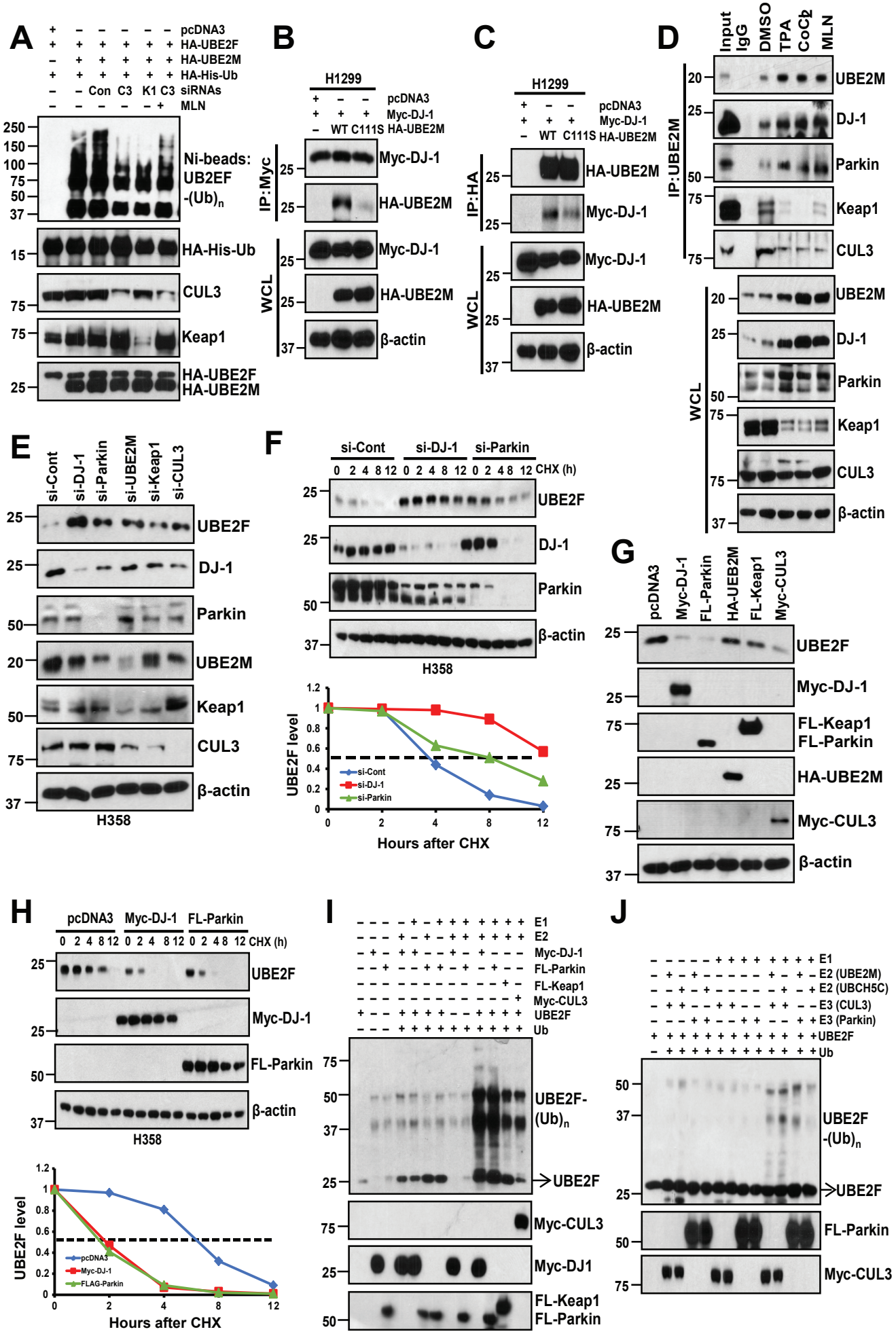
si-Keap1
CUL1
CUL2
CUL3
CUL4A
CUL4B
CUL5
Keap1
β-actin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HA-UBE2M
HA-His-Ub
siRNAs
Con C3 K1 C3
MLN
Ni-beads: UB2EF -(Ub)n
HA-His-Ub
CUL3
Keap1
HA-UBE2F
HA-UBE2M
B
H1299
pcDNA3
Myc-DJ-1
WT C111S HA-UBE2M
IP:Myc
WCL
Myc-DJ-1
HA-UBE2M
β-actin
C
H1299
pcDNA3
Myc-DJ-1
WT C111S HA-UBE2M
IP:HA
WCL
HA-UBE2M
Myc-DJ-1
β-actin
D
Input
IgG
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Parkin
Keap1
CUL3
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E
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Parkin
UBE2M
Keap1
CUL3
β-actin
H358
F
si-Cont
si-DJ-1
si-Parkin
0 2 4 8 12 0 2 4 8 12 0 2 4 8 12 CHX (h)
UBE2F
DJ-1
Parkin
β-actin
H358
UBE2F level
si-Cont
si-DJ-1
si-Parkin
Hours after CHX
G
pcDNA3
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HA-UEB2M
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β-actin
H
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FL-Parkin
0 2 4 8 12 0 2 4 8 12 0 2 4 8 12 CHX (h)
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Myc-DJ-1
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β-actin
H358
UBE2F level
pcDNA3
Myc-DJ-1
FLAG-Parkin
Hours after CHX
I
E1
E2
Myc-DJ-1
FL-Parkin
FL-Keap1
Myc-CUL3
UBE2F
Ub
UBE2F-(Ub)n
UBE2F
Myc-CUL3
Myc-DJ1
FL-Keap1
FL-Parkin
J
E1
E2 (UBE2M)
E2 (UBCH5C)
E3 (CUL3)
E3 (Parkin)
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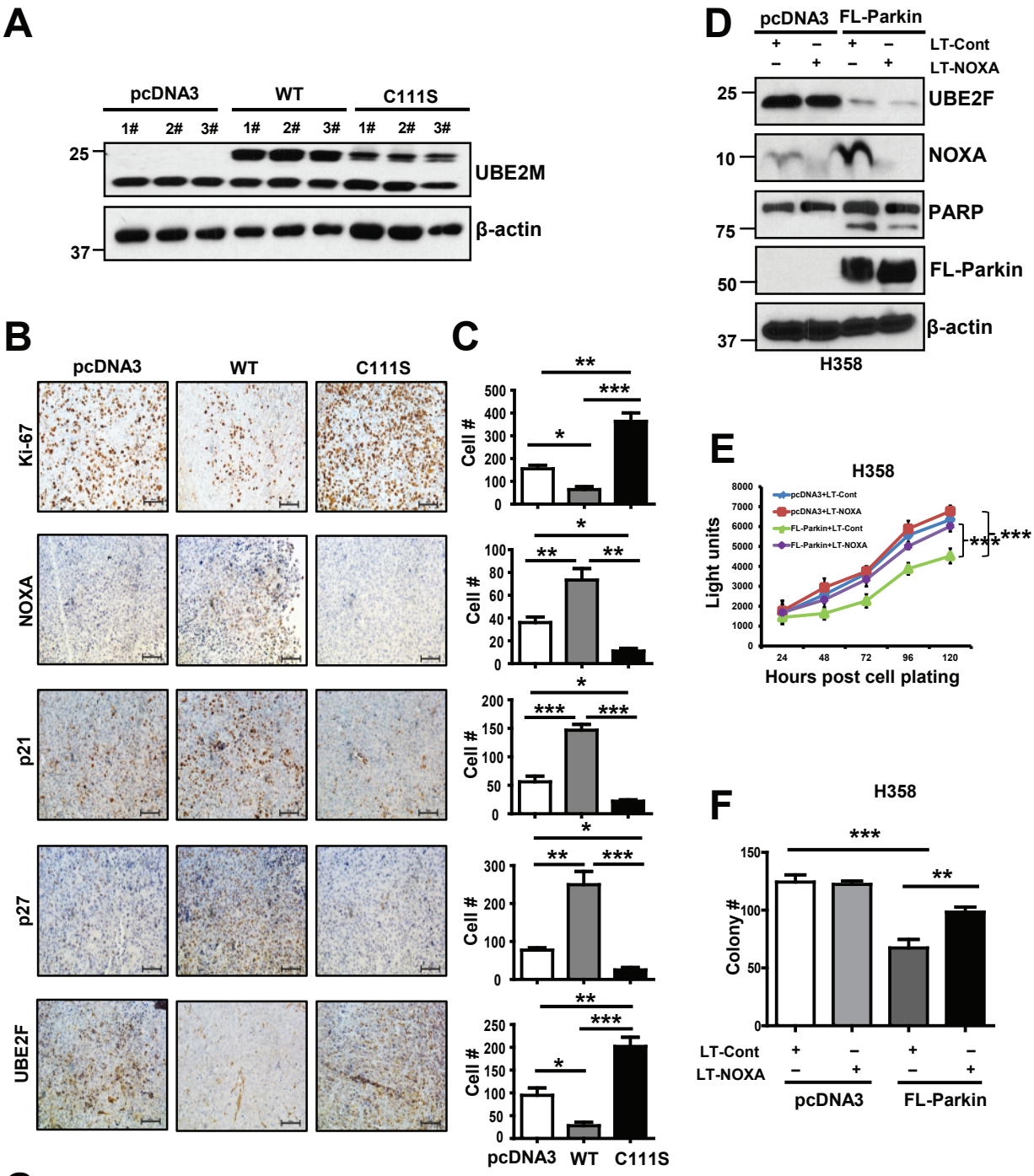
Ub
UBE2F -(Ub)n
UBE2F
FL-Parkin
Myc-CUL3

Zhou et al., Figure S7



G

NSCLC tissues samples		Parkin		UBE2M		Total
		Low	High	Low	High	
UBE2F	Low	17	38	15	40	55
	High	90	13	82	21	103
Total		107	51	97	61	158
$p<0.001, r^2=-0.499$				$p<0.001, r^2=-0.456$		