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Supplemental Material to:

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Loss of VHL promotes progerin expression leading to impaired p14/ARF function and suppression of p53 activity

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Supplementary Figure 1 Nuclear morphology of RCCs. (**a**) DAPI staining of Caki-2 (left) and C2 (right). Nuclear irregularity and lobule formation were clearly detected. (**b**) Nuclear deformation in RCC cell lines. Three types of RCC cell lines were stained with Lamin A/C. (**c**) Information from RCC cell lines. Numbers of references are indicated in the list of supplementary documents. (**d**) Increase of progerin (PG) by G2/M arrest in RCC cell lines. Because of the strong expression of Lamin A (LMA), we did not show the inter-located progerin bands between Lamin A and Lamin C. (**e**) Expression of progerin (PG) in other RCC cell lines. In HGPS (HS) cells, we did not observe the triple bands using Lamin A/C Ab, due to strong expression of Lamin A (LMA).

Supplementary Figure 2 The effect of si-progerin on nuclear deformation of RCC. (**a**) Effect of si-progerin (Si-PG). Si-PG strongly suppressed progerin (PG) expression in HGPS (HS) cell. Among tested Si-PG, based on the effect, we selected # 1 and #2 si-PG for further experiments. (**b**) Si-progerin (Si-PG) can ameliorate the nuclear deformation of HGPS (HS) cells. In the same conditions as Figure 1E, we counted 200 cells and calculated the percentage of nuclear deformed cells. Cell counting was performed by 3 independent researchers. (**c**) Si-progerin (Si-PG) ameliorates the nuclear deformation of RCC cell lines. Cells were transfected with si-PG for 24 hr. Right panel (black and white) shows the DAPI staining of Caki. Left panels shows the staining results of Lamin A/C (green) and DAPI (lower panels; blue) in RCC cells. (**d**) The reduction of nuclear deformation in RCC by si-progerin (Si-PG). After transfection with si-PG or Si-C for 24 hr, we calculated nuclear deformed RCC cells through cell counting, as described above. Numbers are percentages of nuclear deformed cells in each cell line. (**e**) C2 and Caki-2 cells were incubated with 5 μM of FTI (FTI-277) for 24 hr. Cells were fixed with Me-OH and stained with Lamin A/C Ab.

Supplementary Figure 3 pVHL regulates progerin expression in a HIF-1a-independent manner. (**a**) Nuclear morphology of C2 and pVHL stable transfected C2V. Cells were stained with lamin A/C (LMA; green) and DAPI (blue). C2V showed a regular and round nuclear shape. (**b**) Endogenous progerin (PG) is diminished in pVHL stably transfected C2V cells. Compared to C2, progerin expression was decreased

in C2V. Cells were incubated with Noc or Col for 12 hr. (**c**) Knockdown effect of si-VHL. To examine the effect of si-VHL, we measured the expression of pVHL in C2V after transfection of si-VHL (3, 5, and 10 mg/ml). (**d**) pVHL suppresses progerin (PG) expression. Instead, the expression of other Lamin family proteins was not altered by pVHL transfection. A549 cells were co-transfected with pVHL and the indicated GFP-lamin A (LMA), lamin B (LMB), lamin C (LMC) or progerin (PG) vectors for 24 hr. Because we used the GFP-fused vectors, the expression of each gene was examined by the GFP antibody. EV indicates empty-vector transfection. (**e**) Si-pVHL induces progerin (PG) expression. A549 cells were transfected with indicating vectors or siRNA for 24 hr. (**f**) Knockdown effect of si-HIF-1a. To examine the effect of si-HIF1a, we measured the expression of HIF-1a in HIF-1a-transfected 293 cells (5 and 10 mg/ml). (**g**) Si-HIF-1α does not alter progerin expression. To address the effect of HIF-1α, FHIT and pVHL, siRNAs against each gene were transfected in 293 cells and the expression of GFP-lamin A and progerin was measured. Consistent with our data, si-pVHL can increase progerin, whereas si-HIF-1α did not alter the expression of progerin or lamin A. Moreover, the elimination of FHIT evoked the reduction of lamin A and progerin. Until now, the reason for this effect was unclear. (**h**) HIF-1α overexpression does not increase the expression of GFP-progerin (PG). Although HIF-1α overexpression mimics pVHL reduction, it did not induce GFP-PG. Consistent with the previous result, si-HIF-1a did not affect progerin expression. (**i**) HIF-2a does not affect PG expression. 293 cells were co-transfected with si-HIF2a and Lamin family genes for 24 hr.

Supplementary Figure 4 Direct interaction between pVHL and progerin. (**a**) pVHL does not alter the progerin (PG) transcript. In C2, pVHL overexpression did not suppress the progerin expression at the transcription level. This result indicated that pVHL would regulate progerin at the post-transcription level. In fact, we revealed that pVHL reduced the half-life of progerin. (**b**) Densitometric analysis of progerin expression (Fig. 2D). To determine the exact half-life of progerin, we measured the band density using a densitometer. The 20-hr half-life of progerin was shortened to 3.5 hr by pVHL transfection. Conversely, si-VHL extended it up to 24 hr. (**c**) pVHL does not affect the Lamin A half-life. 293 cells were co-transfected with pVHL and lamin A (293-LMA) for 24 hr and incubated with CHX for *de novo* protein synthesis for the indicated time. (**d**) Mutant pVHL cannot ameliorate progerin-induced nuclear deformation.

293 cells were co-transfected with mutant VHL (L158S or R167W) and non-tagged progerin for 24 hr. Differently from wild-type VHL (Fig. 2H), mutant pVHL could not block the progerin-induced nuclear deformation. Cells were fixed with Me-OH and stained with Lamin A/C (green) and HA (red). DAPI staining was used to visualize the DNA. (**e**) VHL can ameliorate the nuclear deformation of HGPS (HS) cells. After transfection with pVHL for 24 hr, we counted the nuclear deformed HS cells. Since Because the transfection efficiency of HGPS is not high, we counted only VHL-positive cells (approximately 100 cells/sample). (**f**) pVHL ameliorates the nuclear deformation of HGPS (HS) cells. After transfection with pVHL, cells were stained with Lamin A/C (LMA; green) and DAPI (blue). (**g**) Interaction of progerin and pVHL. Co-transfected 293 cells with the indicated vectors were subjected into IP analysis. We observed the co-precipitation of progerin (PG) and lamin A (LMA) with FLAG-pVHL. However, the binding between progerin-pVHL seemed to be stronger than that of lamin A-pVHL. (**h**) GST pull-down between GST-pVHL and progerin. To confirm the direct interaction between pVHL-progerin (PG), we generated GST-pVHL and performed the GST pull-down assay. GST-slug was used as a negative control. Considering the fact that lamin C (LMC) did not associate with GST-pVHL, PG-pVHL binding was a specific interaction. (**i**) FTI does not alter the binding between pVHL and progerin or Lamin A. Under the same condition with Fig. S4G, transfected 293 cells were incubated with 5 μM FTI for 4 hr before IP analysis.

Supplementary Figure 5 si-progerin induces p53 in progerin-dependent manner. (**a**) IF staining of p53 in C2 cells. The elimination of p53 inhibitors, including mdm2, COP-1, and Parc, did not induce p53 expression in C2. C2 cells were transfected with the indicated siRNA for 24 hr and stained with p53 (DO-1; green) and DAPI. (**b**) Expression of p53 in normal and progeria cells. Compared to young normal cells (9 years old), p53 expression is reduced in aged (81 years old) or progeria cells (HGPS and WS). (**c**) Elimination of progerin induces p53 in HGPS (HS) cells. Si-progerin (si-PG) could induce p53 and lamin A/C (LMA) in HGPS, while it did not show this effect on normal cells. Moreover, human WRN (H-W) showed a similar effect on p53 and lamin A. In fact, we recently found that human WRN (but not mouse WRN; M-W) can suppress progerin (data not shown). (**d**) Induction of p53 by si-HIF-2a and si-progerin (Si-PG). In VHL-deficient C2, the elimination of progerin or HIF-2a through si-RNA transfection for 24 hr (5 and 10 mg/ml), could induce p53 expression. (**e**) Measurement of apoptosis by cytochrome C (Cyto C)

release. Caki-2 was transfected with si-C or si-progerin (Si-PG) for 24 hr and incubated with 2 mg/ml of Adr for 6 hr. Cells were fixed and stained with cytochrome C antibody. In control cells (Si-C), adriamycin (Adr) did not induce Cyto C release, as it was located in the cytoplasm in a punctured pattern. In contrast, Cyto C was stained in a diffused pattern in si-PG cells in response to Adr. (f) Increase of apoptosis by si-progerin (Si-PG). Based on cytochrome C staining, apoptotic cells were counted (at least 50 cells per sample) and represented as a graph.

Supplementary Figure 6 pVHL inhibits progerin. (a) p53 expression and impact on DNA damage response in RCCs are related to progerin expression. Comparing ACHN and C2V (progerin negative cell lines), p53 expression and impact on adriamycin (Adr) were reduced in C2, A704 and A498. Cells were incubated with 2 mg/ml of Adr for 4 hr. + and – indicate the expression level of progerin in each cell line. (b) p53 is induced by pVHL in Caki-2 cells. Under the same conditions as above, we transfected pVHL into Caki-2 and measured the expression of p53. (c) pVHL does not induce p53 in HCT116. After transfection with pVHL for 24 hr, HCT116 was incubated with 1 mg/ml of adriamycin (Adr) for 2 hr. (d) Si-progerin (Si-PG) does not induce p53 in C2V. In pVHL-transfected C2V, si-PG did not induce p53. C2V cells were transfected with si-PG for 24 hr and incubated with 1 mg/ml of adriamycin (Adr) for 2 hr. (e) Insensitivity of p53 in human progeroid cells. p53 in human progeroid cell lines (HGPS;HS and WS; Werner syndrome cell line) was not induced by adriamycin (Adr) treatment. However, normal fibroblasts responded to it. In addition, both cell lines showed a low level of Lamin A/C. Cells were incubated with the indicated concentration of Adriamycin (Adr) for 4 hr. (f) Synergistic effect of p14/ARF and si-Progerin (Si-PG). In HGPS (HS), the elimination of progerin could induce p53 in response to adriamycin (Adr). Furthermore, co-transfection with p14 could synergistically induce p53. (g) Overexpression of p14 can overcome progerin (PG)-induced p53 suppression. HCT116 was transfected with indicating vectors for 24 hr and incubated with adriamycin (Adr) or camptothecin (CPT) for 2 hr. p21 expression was used for monitoring the activity of p53. (h) Effect of si-p14. To evaluate the knockdown effect of p14, we measured the expression of p14 after transfection with si-p14 (3, 5, and 10 mg/ml) into p14-GFP-transfected 293 cells.

Supplementary Figure 7 p14 does not amend progerin-induced nuclear deformation. (a) p14/ARF does not affect the nuclear morphology. In HGPS (HS) cells, we transfected p14/ARF alone or with pVHL or si-progerin (Si-PG) and checked the nuclear morphology using IF with Lamin A/C antibody (LMA; green). p14/ARF alone could not ameliorate the nuclear deformation. Instead, pVHL and si-PG could block the nuclear deformation. (b) Cell counting result. Nuclear deformation is not affected by p14 overexpression.

Supplementary Figure 8 Schematic summary. (a) Expression of progerin (PG) in leukemia cell lines. Among three newly established cell lines, the SJW series showed the expression of progerin (PG), which is eliminated by si-PG. (b) Diagram for Summary. Under aging conditions, progerin is accumulated. However, pVHL eliminates progerin. In RCCs (pVHL deficient condition), progerin may be rapidly accumulated and block p14. In addition, elevated HIF-2a would contribute to p53 inactivation. Therefore, p53 does not properly function and cancer easily occurs.

Supplementary Table 1 Clinical information of leukemia samples. Asterisk (*) indicates the expression level of progerin and lamin A at transcription (RT-PCR)

#	Age	Gender	Diagnosis	Description	Expression Level	
					Lamin A/C	Progerin
1	38	M	AML-M1	Acute myeloid leukemia without maturation, M1	5	5*
2	57	F	ALL-remission	Acute lymphoblastic leukemia in remission	5	5
3	44	F	AML-M1	Acute myeloid leukemia without maturation, M1 in regenerating marrow with engraftment. Allo SCT 14days	5	5
4	29	M	AML-M1	Acute myeloid leukemia without maturation(M1) in regenerating	5	5
5	21	F	ALL	Acute lymphoblastic leukemia, B lymphoblastic leukemia in regenerating marrow without residual leukemia, f/u, see above. Chemotherapy 21 days	5	5
6	72	M	AML-M3	Acute promyelocytic leukemia, microgranular type, M3 in recurrence	5	5
7	38	F	AML-M1	Acute myeloid leukemia, without maturation, FAB(M1) in recurrence	5	5
8	36	M	AML-M1	Acute myeloid leukemia without maturation (M1) in remission	5	5
9	66	F	AML-M1	Acute myeloid leukemia without maturation (M1), with t(6:11), MLLT4-MLL, refer to RT-PCR	5	5
10	72	M	AML-M4	Acute myelomonocytic leukemia in regenerating granulocytic hyperplasia with a few residual leukemic cells. f/u, chemotherapy 28 days	5	5
11	29	F	ALL-B	Acute lymphoblastic leukemia, B lymphoblastic leukemia	0	0
12	66	M	AML-M4	Acute myelomonocytic leukemia	4	0
13	44	F	AML-M1	Acute myeloid leukemia without maturation, M1	5	0
14	41	F	AML-M3	Acute promyelocytic leukemia with PML-RARa	2	0
15	37	M	AML-M3	Acute promyelocytic leukemia, microgranular	0	0
16	57	F	ALL-B	Acute lymphoblastic leukemia, B lymphoblastic leukemia(WHO)	0	0
17	26	M	NORMAL	Normocellular marrow without tumor involvement	1	0
18	45	F	NORMAL	Nomocellular marrow, otherwise not specific	2	0
19	49	M	NORMAL	Nomocellular marrow without myeloma involvement, f/u	3	0

References for Supplementary Figure 1

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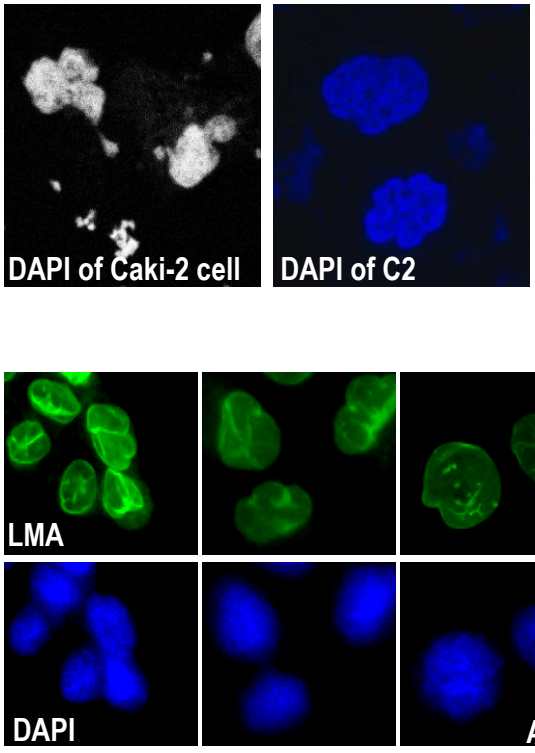
A



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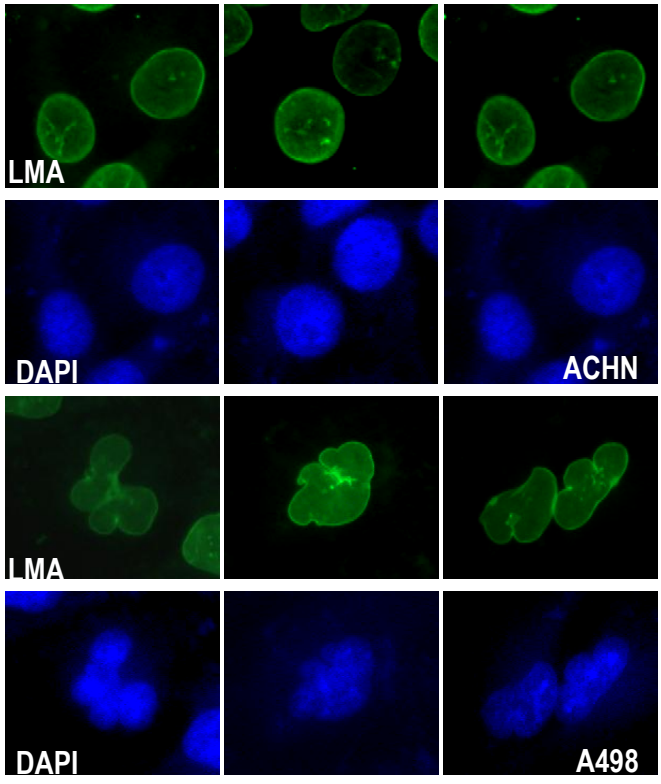
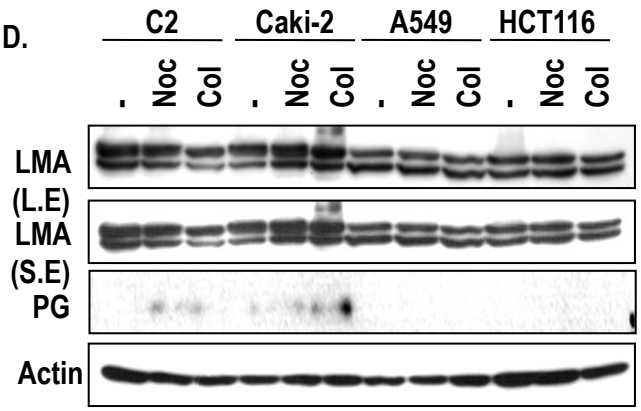


C.

Cell line	gender	age	P53 status	pVHL status	Nuclear deformation	references
Caki-2	Male	69	WT	Mutant	67%	1, 2
C2	unknown	unknown	WT	null	86	3
ACHN	male	22	WT	WT	4	2, 4, ATCC
A498	Female	52	WT	null	65	2, 4, ATCC
A704	male	78	WT	Mutant (germ line)	54	2, 4, ATCC

D.



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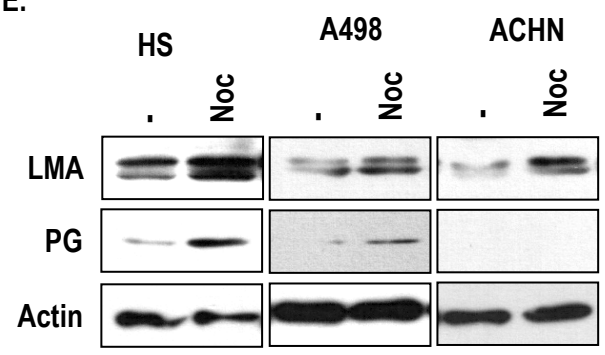
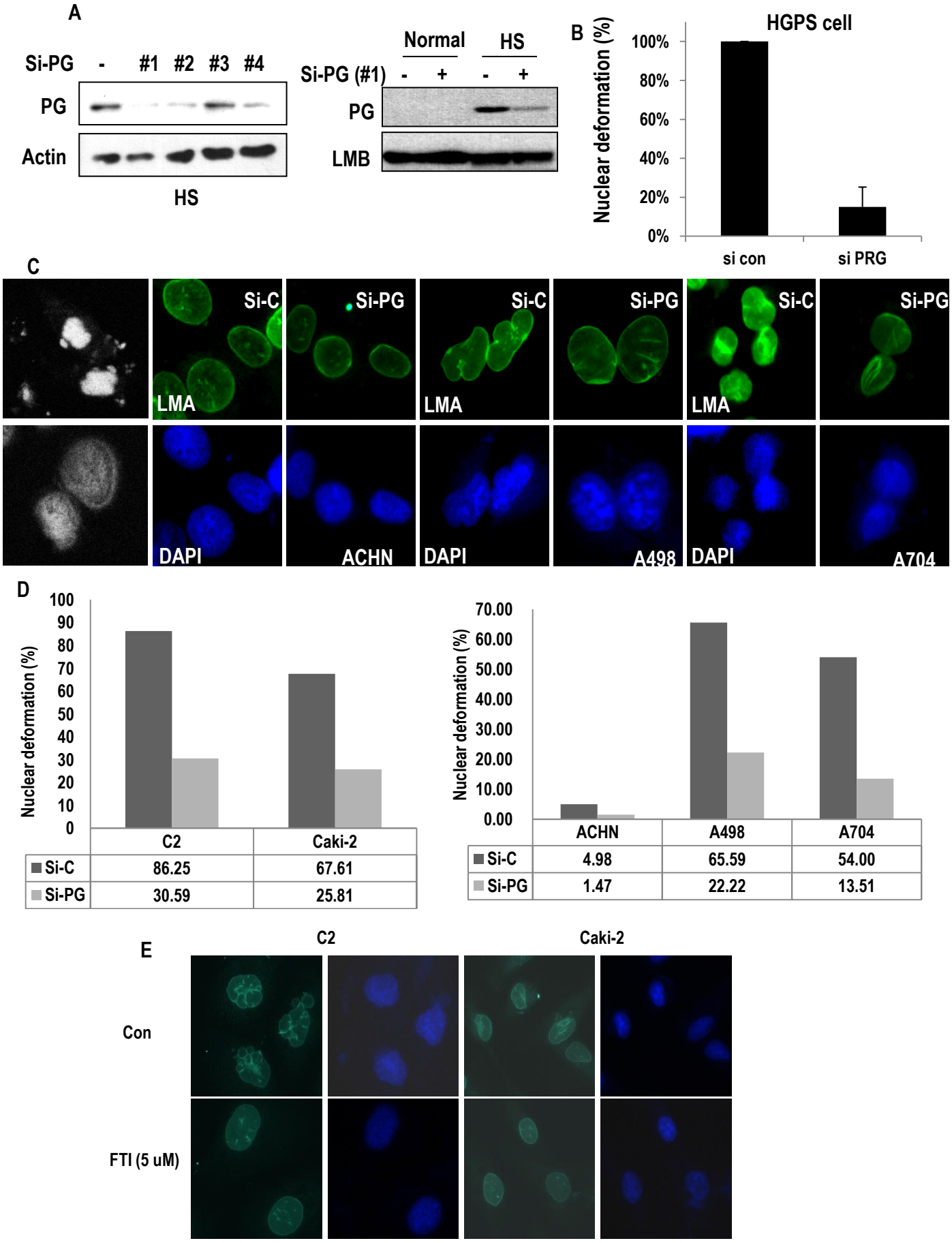
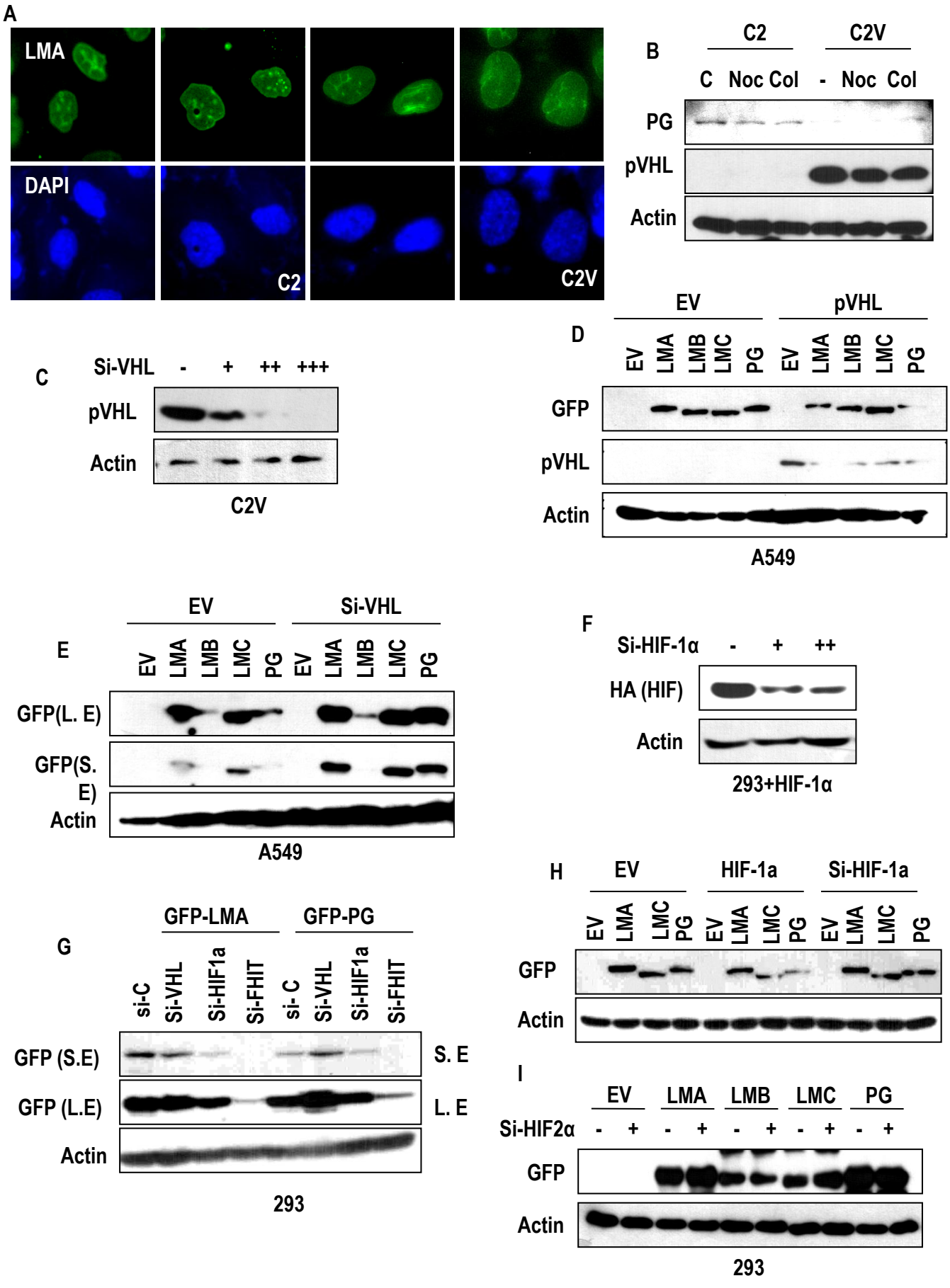
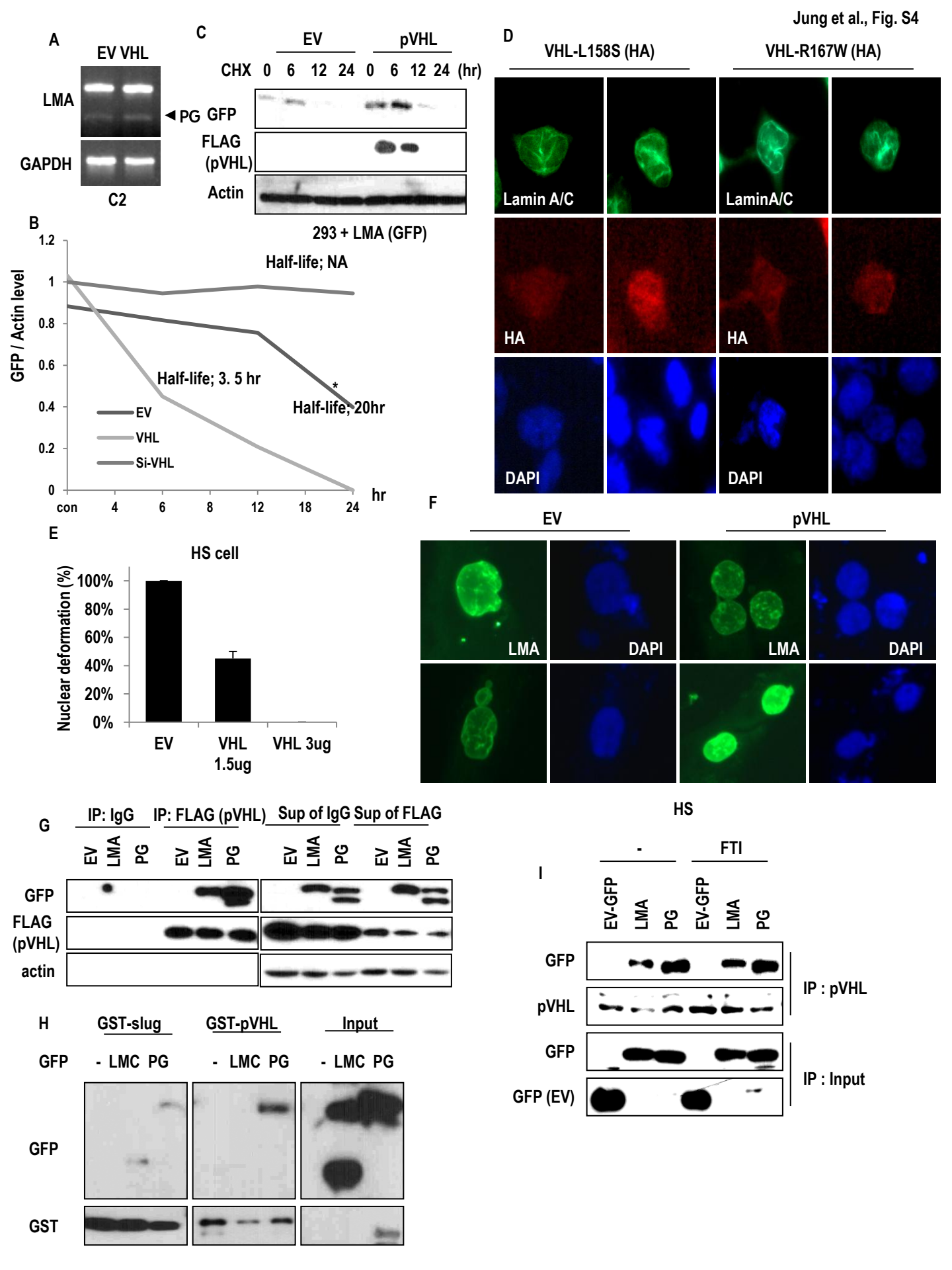
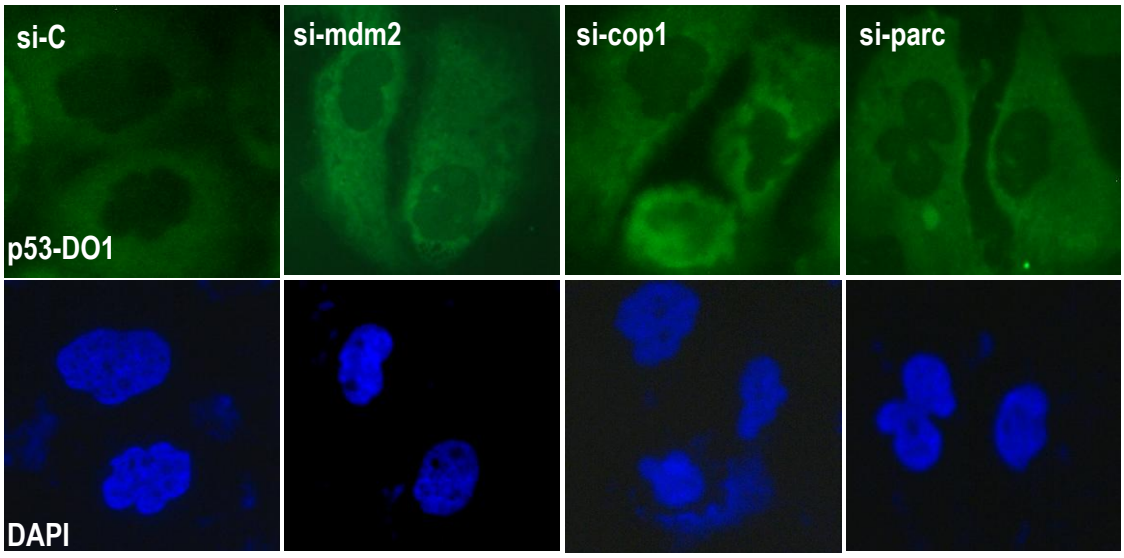



A
Si-PG - #1 #2 #3 #4
PG
Actin
HS
Normal HS
Si-PG (#1) - + - +
PG
LMB
B
HGPS cell
Nuclear deformation (%)
100%
80%
60%
40%
20%
0%
si con
si PRG
C
Si-C
Si-PG
LMA
DAPI
ACHN
A498
A704
D
Nuclear deformation (%)
C2
Caki-2
Si-C 86.25 67.61
Si-PG 30.59 25.81
ACHN
A498
A704
Si-C 4.98 65.59 54.00
Si-PG 1.47 22.22 13.51
E
C2
Caki-2
Con
FTI (5 uM)

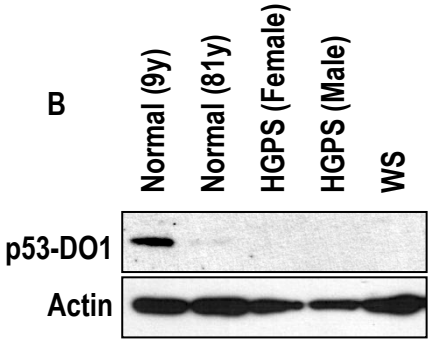

A
LMA
DAPI
C2
C2V
B
C2
C2V
C Noc Col - Noc Col
PG
pVHL
Actin
C
Si-VHL - + ++ +++
pVHL
Actin
C2V
D
EV
pVHL
EV LMA LMB LMC PG EV LMA LMB LMC PG
GFP
pVHL
Actin
A549
E
EV
Si-VHL
EV LMA LMB LMC PG EV LMA LMB LMC PG
GFP(L. E)
GFP(S. E)
Actin
A549
F
Si-HIF-1α - + ++
HA (HIF)
Actin
293+HIF-1α
G
GFP-LMA
GFP-PG
si-C Si-VHL Si-HIF1a Si-FHIT si- C Si-VHL Si-HIF1a Si-FHIT
GFP (S.E)
GFP (L.E)
Actin
S. E
L. E
293
H
EV
HIF-1a
Si-HIF-1a
EV LMA LMC PG EV LMA LMC PG EV LMA LMC PG
GFP
Actin
I
EV
LMA
LMB
LMC
PG
Si-HIF2α - + - + - + - + - +
GFP
Actin
293


A
EV VHL
LMA
GAPDH
C2
C
EV
pVHL
CHX 0 6 12 24 0 6 12 24 (hr)
PG GFP
FLAG (pVHL)
Actin
B
293 + LMA (GFP)
Half-life; NA
Half-life; 3. 5 hr
Half-life; 20hr
EV
VHL
Si-VHL
GFP / Actin level
hr
D
VHL-L158S (HA)
VHL-R167W (HA)
Lamin A/C
LaminA/C
HA
DAPI
E
HS cell
Nuclear deformation (%)
EV
VHL 1.5ug
VHL 3ug
F
EV
pVHL
LMA
DAPI
G
IP: IgG
IP: FLAG (pVHL)
Sup of IgG
Sup of FLAG
EV LMA PG
GFP
FLAG (pVHL)
actin
H
GST-slug
GST-pVHL
Input
GFP - LMC PG
GST
HS
I
-
FTI
EV-GFP LMA PG
GFP
pVHL
IP : pVHL
GFP (EV)
IP : Input

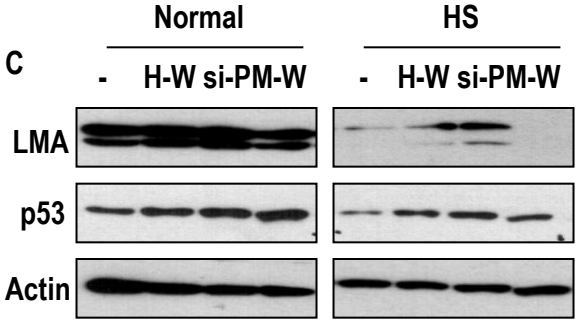
A


si-C
si-mdm2
si-cop1
si-parc
p53-DO1
DAPI

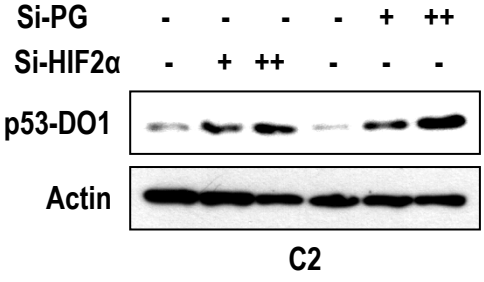
B


Normal (9y)
Normal (81y)
HGPS (Female)
HGPS (Male)
WS
p53-DO1
Actin

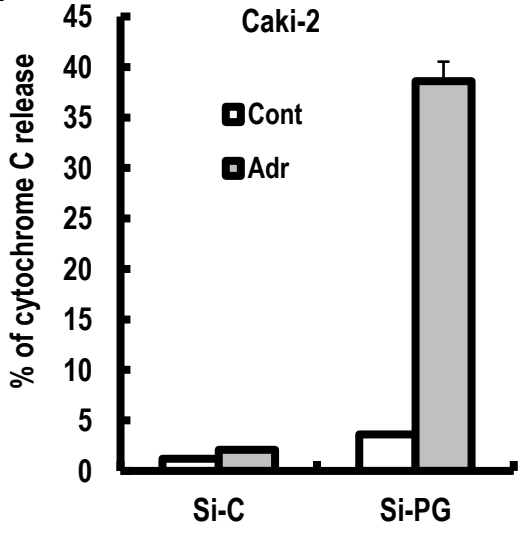
C


Normal
HS
- H-W si-PM-W
- H-W si-PM-W
LMA
p53
Actin

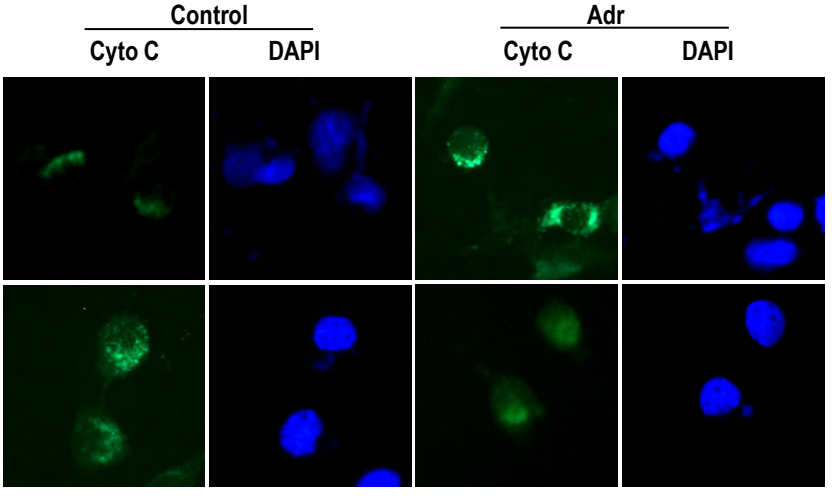
D

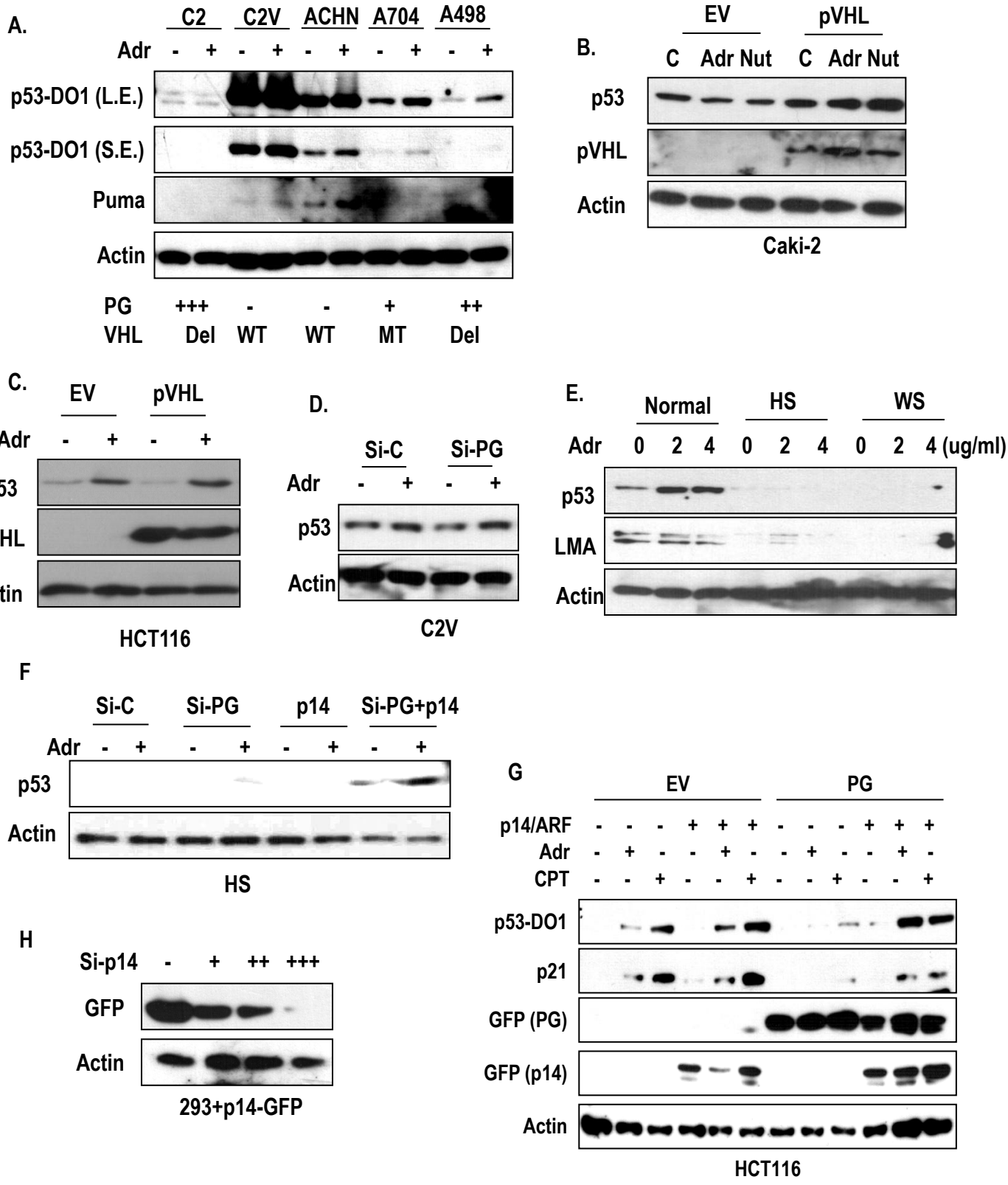

Si-PG - - - - + ++
Si-HIF2α - + ++ - - -
p53-DO1
Actin
C2

E

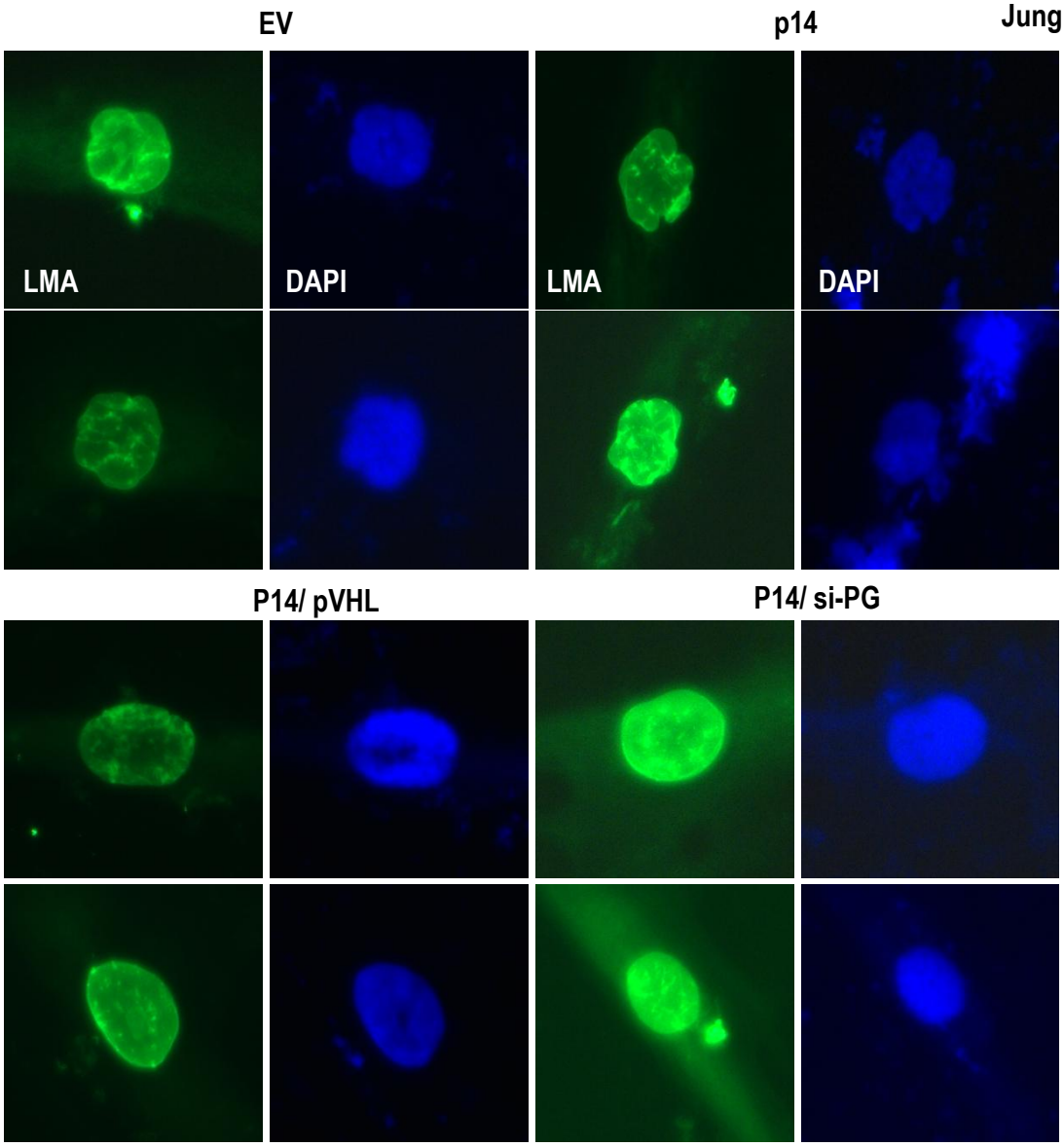

Caki-2
Cont
Adr
% of cytochrome C release
45
40
35
30
25
20
15
10
5
0
Si-C
Si-PG

F

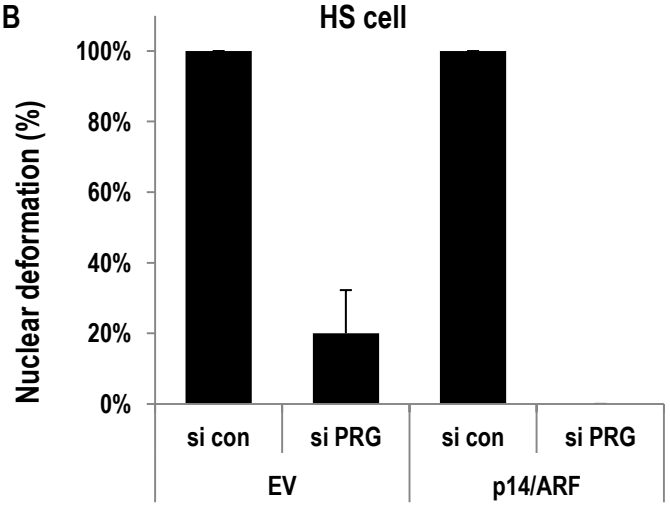

Control
Adr
Cyto C
DAPI
Cyto C
DAPI
Si-C
Si-PG


A.
C2
C2V
ACHN
A704
A498
Adr - + - + - + - + - +
p53-DO1 (L.E.)
p53-DO1 (S.E.)
Puma
Actin
PG +++ - - + ++
VHL Del WT WT MT Del
B.
EV
pVHL
C Adr Nut C Adr Nut
p53
pVHL
Actin
Caki-2
C.
EV
pVHL
Adr - + - +
p53
pVHL
Actin
HCT116
D.
Si-C
Si-PG
Adr - + - +
p53
Actin
C2V
E.
Normal
HS
WS
Adr 0 2 4 0 2 4 0 2 4 (ug/ml)
p53
LMA
Actin
F
Si-C
Si-PG
p14
Si-PG+p14
Adr - + - + - + - +
p53
Actin
HS
G
EV
PG
p14/ARF - - - + + + - - - + + +
Adr - + - - + - - + - - + -
CPT - - + - - + - - + - - +
p53-DO1
p21
GFP (PG)
GFP (p14)
Actin
HCT116
H
Si-p14 - + ++ +++
GFP
Actin
293+p14-GFP

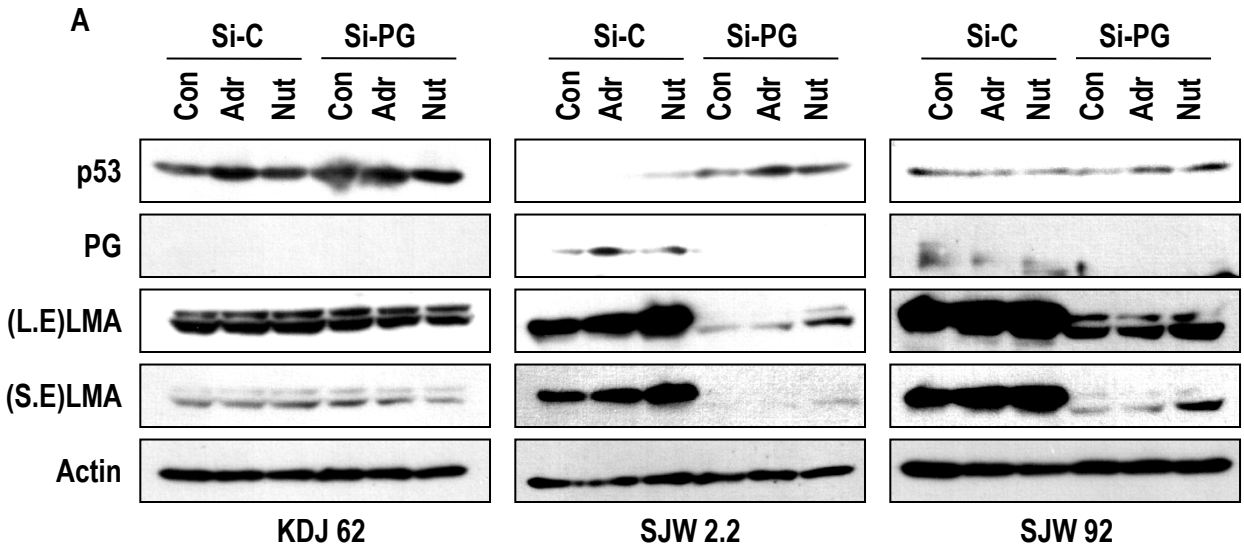
A


EV
p14
LMA
DAPI
LMA
DAPI
P14/ pVHL
P14/ si-PG

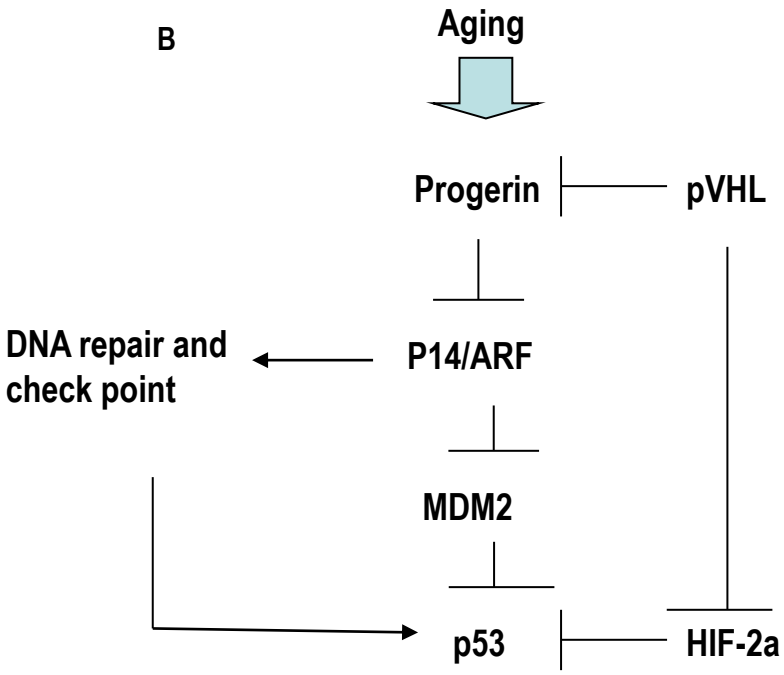
B


HS cell
Nuclear deformation (%)
100%
80%
60%
40%
20%
0%
si con
si PRG
si con
si PRG
EV
p14/ARF

A


Si-C
Si-PG
Con
Adr
Nut
p53
PG
(L.E)LMA
(S.E)LMA
Actin
KDJ 62
SJW 2.2
SJW 92

B


Aging
Progerin
pVHL
DNA repair and
check point
P14/ARF
MDM2
p53
HIF-2a