Supplementary data

Supplementary Figure 1. PRR11 shows meshwork structure

(**a**) WT PRR11 expression exhibited meshwork distribution. Cells were stained with flag (green) and DAPI (blue) for the identification of Flag-PRR11 overexpression. Bars, 20 μm. (**b**) Overexpression of WT PRR11 recruits Arp2. H1299 cells were transfected with pvN173 Flag-control expressing construct or Flag-WT PRR11 construct. Cells were fixed and stained for Flag and the Arp2 antibody. White arrowhead indicates WT PRR11-expressing cells and red arrowhead shows pvN173-expressing cells, and yellow arrow shows endogenous Arp2 localizes at membrane protrusion. Cell nuclei were stained with DAPI (blue). Scale bars, 20 μm. Zoomed images of boxed region are shown on the right panels. Representative images are shown (Scale Bars, 2 μm). (**c**) Co-localization analysis of fluorescence signal in Flag-positive cells from (**b**) was performed. The line graphs represent overlap between Arp2 and Flag-control signal (left panel) or Flag-PRR11 signal (right panel) at the region of interest (the white line from (**b**)). (**d**) Relative intensity of Arp2 immunofluoresence was statistically quantified in the images of pvN173 and Flag-PRR11 expressing cells. Results were shown as the mean ±SEM (n = 3). Greater than 10 cells were counted per condition in every repeat. (**e**) Antibodies against PRR11 and Flag identify exogenous Flag-PRR11. H1299 cells were stained with anti-PRR11 (red), anti-flag (green) and DAPI (blue) after 24 hours of Flag-PRR11 transfection. Scale Bars, 20 μm. Zoomed images of boxed region are shown on the down panels (Scale Bars, 2 μm).

Supplementary Figure 2. Schematic of PRR11 mutated

(**a**) Schematic representation of different mutated forms of PRR11. (**b**) The effects of Δ33-41 on F-actin polymerization and rearrangement. H1299 cells were overexpressed with Flag-tagged Δ33-41, and then cells were fixed 24 hours after and stained for Flag and phalloidin. Representative images were shown. Bar, 10 μm.

Supplementary Figure 3. Aberrant high PRR11 expression led to disordered nuclear lamina assembly

(**a**) High expression level of endogenous PRR11 relates with irregular nuclear lamina. H1299 cells were stained with PRR11 and Lamin A/C. Red and white asterisk indicate the PRR11-high and PRR11-low expression cells, respectively. Bars, 10 μm. (**b**) H1299 cells treated with siNC or siPRR11 were stained for lamin A/C. Representative images were shown. White arrowheads indicated the cells with irregular shape. Bars, 10 μm. (**c**) Quantification of cells of irregular shape demonstrated in (**b**). Results were shown as the mean ±SEM. (n =3), Greater than 50 cells were counted per condition in every repeat. (**d**) Immunofluorescence staining for lamin A/C and Flag in the cells transfected with Flag-tagged pVN173 or Flag-PRR11. White arrowheads indicated that the localization of lamin A/C was highly disordered in Flag-PRR11 expressing cells. Representative images were shown. Bars, 10 μm. (**e**) Quantification of cells with disordered lamin A/C as normalized to total Flag-positive cells demonstrated in (**d**). Results were shown as the mean ±SEM. (n =3), Greater than 50 cells were counted per condition in every repeat. (**f**) and (**g**) Co-localization analysis for endogenous PRR11 and Lamin A/C. (**f**) H1299 cells were stained with PRR11 (red) and Lamin A/C (green). Bars, 5 μm. (**g**) Co-localization analysis of fluorescence intensity in the cells from (**f**). The line graphs represent overlap between Lamin A/C and PRR11 signal at the region of interest (the white line from **f**). (**h**) The subcellular localization of PRR11 in extracts. The cytoplasmic and nuclear lysates from cells were analyzed by western blotting with antibodies against indicated proteins. Cell nuclei were stained with DAPI (blue). ***$p < 0.001$.

Supplementary Figure 4. Ectopic expression of PRR11 induces abnormal chromatin organization

(a) Overexpression PRR11 induces abnormal chromatin organization. H1299 and A549

cells were transiently transfected with pvN173 or Flag-PRR11 and then fixed and stained with anti-Flag 24 h after transfection. Cell nuclei were stained with DAPI (blue). Representative confocal images are shown. Bars, 20 μm. The nuclear showed normal morphology in pvN173-expressing cells. However, the whole nucleus showed irregular condensation in WT PRR11 expressing cells (green arrowheads). **(b)** Quantification of DAPI fluorescence intensity normalized to nuclear area in Flag-positive cells proved that Flag-PRR11 overexpression notably driven chromatin organization compared with control pvN173 (Flag-tagged N-terminal 173 residues of eGFP)**.** Results are shown as the mean ±SEM. (n > 50). **(c)** Quantification of abnormal chromatin organization cells normalized to total Flag-positive cells from **(a).** Results are shown as the mean ±SEM. (n =3). Greater than 100 cells were counted per condition in every repeat. **(d)** Quantitative PCR was performed to show knock-down of protein expression in cells treated for 48h with condensin I (CAP-E, CAP-D2, CAP-G) and topoisomerase IIa (topo IIa)-targeted siRNA, either individually or in combination. Bars represent ±SEM. **(e)** H1299 and A549 cells exposure to siNC or siRNA targeted chromatin condensation-related mRNA was transiently transfected Flag-PRR11. And then cells were fixed and stained with Flag and DAPI. Quantification of percentage of nuclei that score as abnormal chromatin organization in Flag-PRR11 expressing cells. Representative histogram images are shown. Results are shown as the mean ±SEM. (n= 3). Greater than 50 cells were counted per condition in every repeat, contained in greater than or equal to three fields. **(f)** Abnormal chromatin organization results in apoptosis in WT PRR11–expressing cells. Cells were transiently transfected with eGFP or Flag-PRR11. Cells were fixed and stained with anti-Flag (green) and anti-cleaved Caspase 3 (red). Cell nuclei were stained with DAPI (blue). White arrowheads indicates irregular nuclear. Red arrowheads indicate apoptotic cells. Scale bars, 20 μm. **(g)** Quantitative analysis of the effect demonstrated in (**f).** Quantification of cells of cleaved caspase 3 positive cells and irregular nucleus in Flag-positive cells. Results are shown as the mean ±SEM. (n= 3). Greater than 50 cells were counted per condition in every repeat,

contained in greater than or equal to three fields. ***$p < 0.001$, N.S ($p > 0.05$).

Supplementary Figure 5. Silencing of Lamin B1 abolishes PRR11 overexpression induced heterochromatin reorganization

(a) Overexpression of PRR11 disorders H3K4me3. White arrowheads indicate decreased H3K4me3 expression in PRR11-expressing cells. Bar, 20 μm. **(b)** Quantification of cells of disordered H3K4me3 normalized to total Flag-positive cells demonstrated in **(a)**. Results are shown as the mean ±SEM. (n=3). Greater than 50 cells were counted per condition in every repeat, contained in greater than or equal to three fields. (**c**) Western blot analysis for the indicated proteins. H1299 and A549 cells treated with siNC or siRNA directed against Lamin B1 were lysed 48 h after transfection, and analyzed for expression of the indicated proteins by western blotting. (**d**) Lamin B1 knockdown led to the redistribution of H3K9me3 heterochromatin. Cells were treated as (**c**). Cells were fixed 48 h after transfection and stained for H3K9me3. Representative images were shown. Bars, 20 μm. Zoomed images of boxed region are shown at the left-bottom corner (Scale Bars, 10 μm). (**e**) Qualification of cells with H3K9me3 nuclear peripheral localization in siNC and siLamin B1 cells. Results are shown as the mean ±SEM. (n= 3). Greater than 50 cells were counted per condition in every repeat. (**f**) and (**g**) Cells with diminished H3K9me3 nucleoplasm localization in H1299 cells overexpressed with WT PRR11 with or without Lamin B1 knockdown. H1299 cells were depleted Lamin B1 via transfection siRNA 24 h after, and then cells were fixed 24 hours after transfection and stained for Flag and H3K9me3. Representative images were shown. Bar, 10 μm. (**g**) Quantification of cells with diminished H3K9me3 nucleoplasm localization in H1299 cells overexpressed with WT PRR11 with or without Lamin B1 knockdown. Results were shown as the mean ±SEM. (n=3). Greater than 50 cells were counted per condition in every repeat. (**h**) Cells were lysed, and analyzed for expression of the indicated proteins by western blotting of whole-cell lysates. Cell nuclei were stained with DAPI (blue). ***$p < 0.001$.

Supplementary table 1. Oligonucleotides used in the present study

Supplementary table 2. Antibodies used in the present study

Figure S1

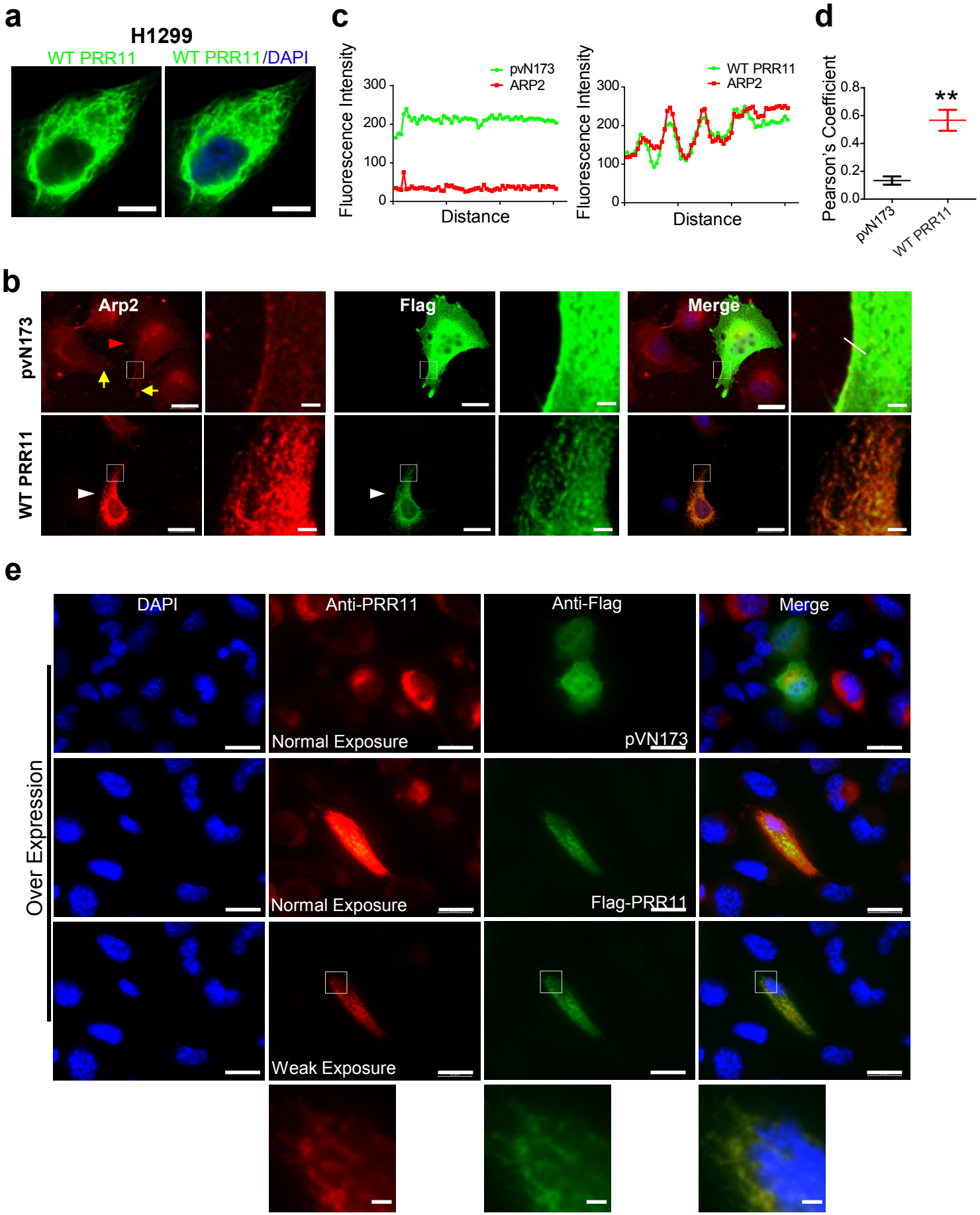
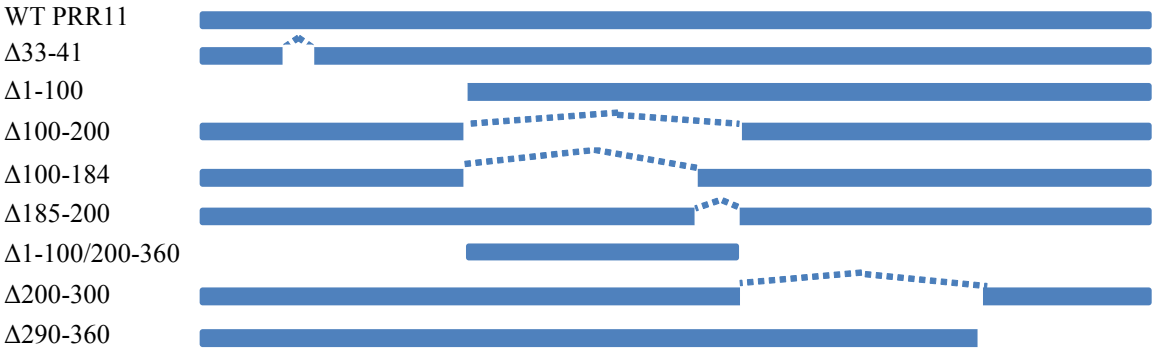

a
H1299
WT PRR11
WT PRR11/DAPI
c
Fluorescence Intensity
pvN173
ARP2
Distance
WT PRR11
ARP2
d
Pearson's Coefficient
**
pvN173
WT PRR11
b
pvN173
WT PRR11
Arp2
Flag
Merge
e
Over Expression
DAPI
Anti-PRR11
Anti-Flag
Merge
Normal Exposure
pVN173
Flag-PRR11
Weak Exposure

Figure S2

a


WT PRR11
Δ33-41
Δ1-100
Δ100-200
Δ100-184
Δ185-200
Δ1-100/200-360
Δ200-300
Δ290-360

b

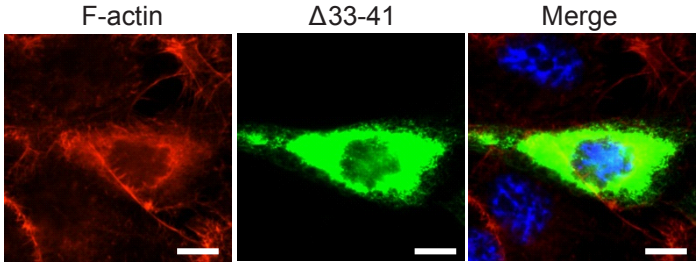

F-actin
Δ33-41
Merge

Figure S3

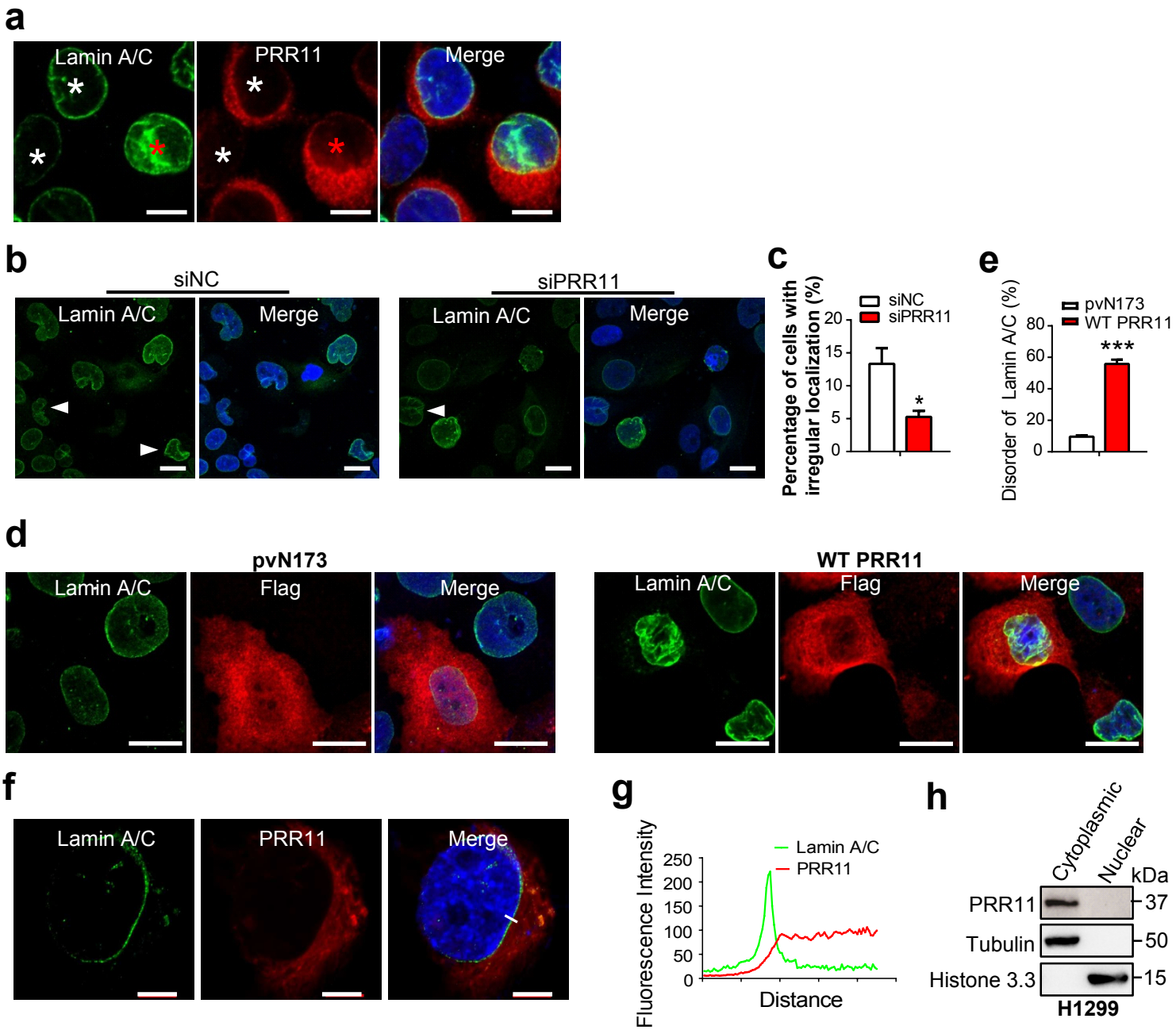

a
Lamin A/C
PRR11
Merge
b
siNC
siPRR11
Lamin A/C
Merge
c
Percentage of cells with irregular localization (%)
siNC
siPRR11
e
Disorder of Lamin A/C (%)
pvN173
WT PRR11
***
d
pvN173
WT PRR11
Lamin A/C
Flag
Merge
f
g
Fluorescence Intensity
Distance
h
Cytoplasmic
Nuclear
kDa
PRR11
Tubulin
Histone 3.3
H1299

Figure S4

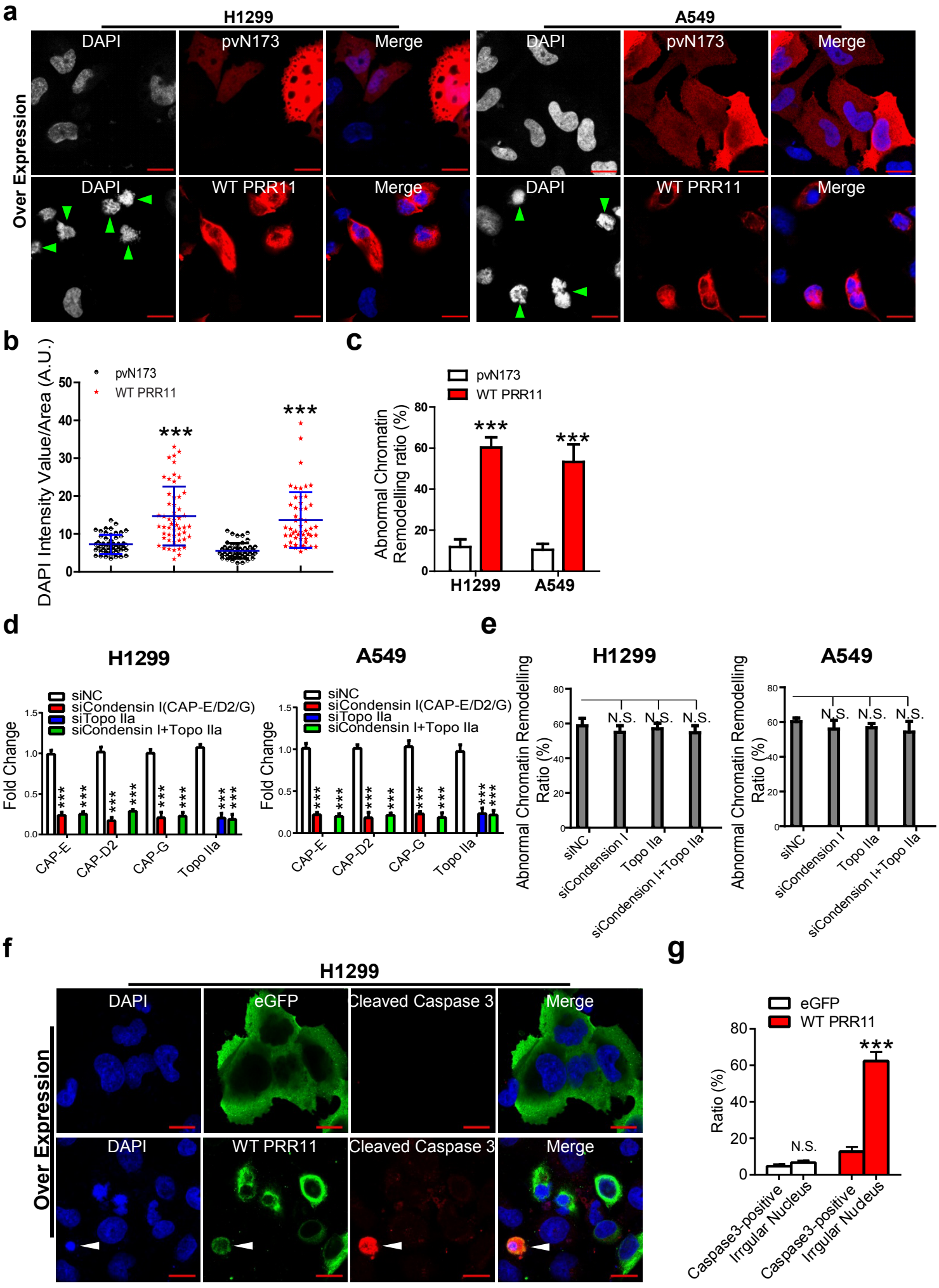
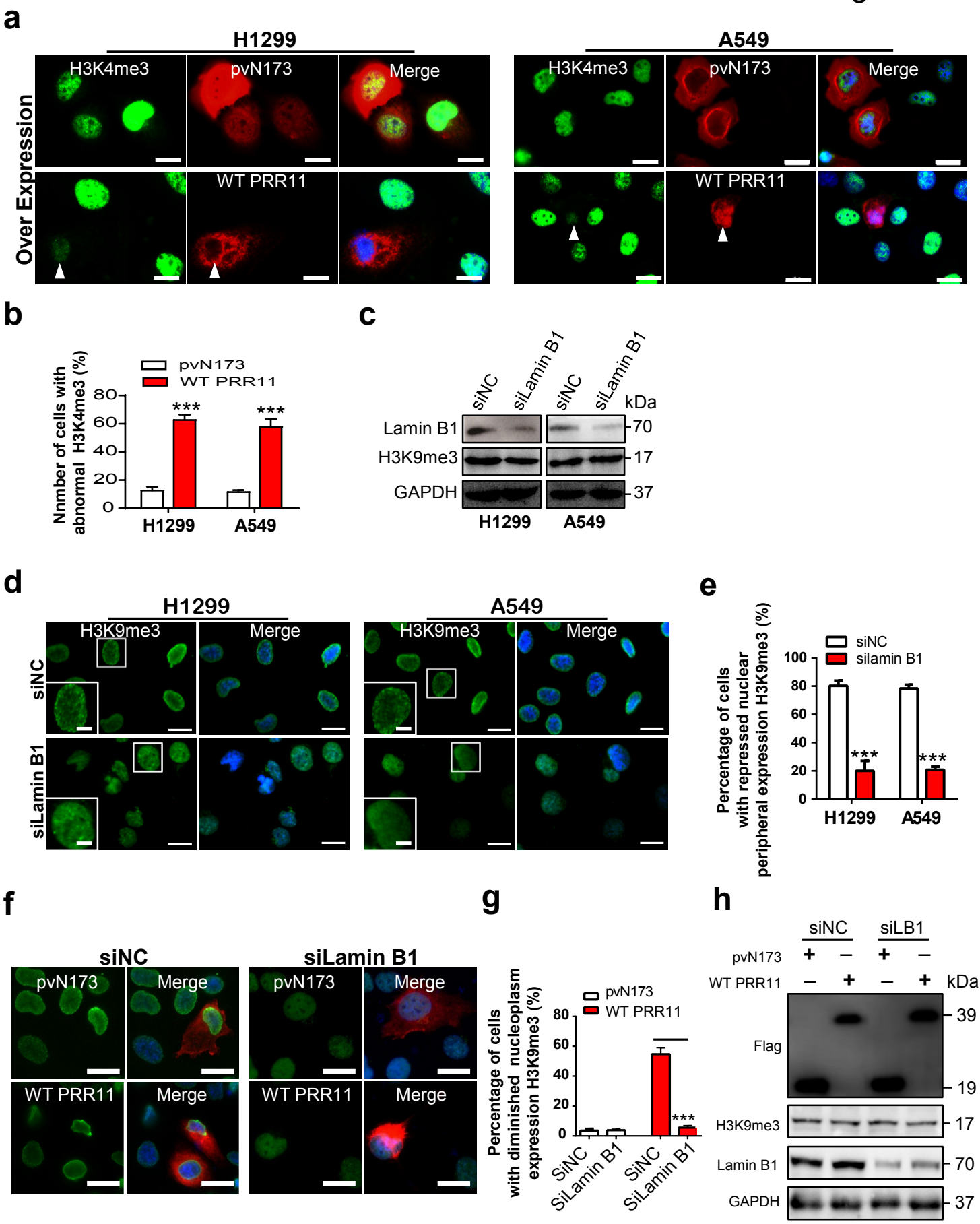

a
H1299
A549
DAPI
pvN173
Merge
WT PRR11
Over Expression
b
DAPI Intensity Value/Area (A.U.)
pvN173
WT PRR11
***
c
Abnormal Chromatin Remodelling ratio (%)
H1299
A549
d
H1299
A549
siNC
siCondensin I(CAP-E/D2/G)
siTopo IIa
siCondensin I+Topo IIa
Fold Change
CAP-E
CAP-D2
CAP-G
Topo IIa
e
Abnormal Chromatin Remodelling Ratio (%)
N.S.
siNC
siCondension I
Topo IIa
siCondension I+Topo IIa
f
H1299
Over Expression
DAPI
eGFP
Cleaved Caspase 3
Merge
WT PRR11
g
eGFP
WT PRR11
Ratio (%)
N.S.
Caspase3-positive Irrgular Nucleus

Figure S5


a
H1299
A549
H3K4me3
pvN173
Merge
WT PRR11
Over Expression
b
Nnmber of cells with abnormal H3K4me3 (%)
pvN173
WT PRR11
80
60
40
20
0
***
***
H1299
A549
c
siNC
siLamin B1
siNC
siLamin B1
kDa
Lamin B1
H3K9me3
GAPDH
70
17
37
H1299
A549
d
H1299
A549
H3K9me3
Merge
siNC
siLamin B1
e
Percentage of cells with repressed nuclear peripheral expression H3K9me3 (%)
siNC
silamin B1
100
80
60
40
20
0
***
***
H1299
A549
f
siNC
siLamin B1
pvN173
Merge
WT PRR11
g
Percentage of cells with diminished nucleoplasm expression H3K9me3 (%)
pvN173
WT PRR11
80
60
40
20
0
***
SiNC
SiLamin B1
SiNC
SiLamin B1
h
siNC
siLB1
pvN173
WT PRR11
kDa
Flag
H3K9me3
Lamin B1
GAPDH
39
19
17
70
37

Supplementary table 1. Oligonucleotides used in the present study

siRNA

PRR11	
ACG CAG GCC UUA AGG AGA ATT	UUC UCC UUA AGG CCU GCG UTT
ARPC1A	
AUCACGAAGUGCACAUCUATT	UAGAUGUGCACUUCGUGAUTT
Lamin B1	
CGCGCUUGGUAGAGGUGGATT	UCCACCUCUACCAAGCGCGTT
Topo IIa	
5-GGUAUUCCUGUUGUUGAACtt-3	5-GUUCAACAACAGGAAUACCtt-3
CAP-E	
5-UGCUAUCACUGGCUUAAAUTT-3	5-CAUAUUGGACUCCAUCUGCTT-3
CAP-G2	
5-UGAUUGCAUCCAGGACUUCTT-3	5-CUCUGAAGUUCGAUCAAAUTT-3
CAP-D2	
5-UCAGUAUGUUGUGCAAGAGTT-3	5-GAAGAUACUCUGGAAUUCCTT-3

Primers used for PCR

PRR11-pET-GST F 5'cgcGGATCCATGGACTACAAGGACGACGA 3'
PRR11-pET-GST R 5'ccgCTTAAGTCAGTTTTGTTCATCAAAGCTG 3'
PRR11-1-R 5' CTTGTCATCGTCGTCCTTGT 3'
PRR11-33-R 5' AATTAGCTTGGACTGAAAGTGAG 3'
PRR11-41-F 5' GAAAGAGTCGGTATTTCTTCAAT 3'
PRR11-100-F 5' ACCATCTTTCCATCTCGTAT 3'
PRR11-100-R 5' GTCTTTCAATACTTCTAAACTC 3'
PRR11-184-F 5' CCTCCTCCTCCTCCACCTCCACCTC 3'
PRR11-185-R 5' ATGGCTGGCTGGCTGTGGC 3'
PRR11-200-F 5' GCACCTGTGTTGCTCAGAAAACC 3'
PRR11-200-R 5' TAGTGGTGGTGGAGGAGGTGGC 3'
PRR11-290-R 5' TTTTCCAGGAGTGATTAAGACG 3'
PRR11-300-F 5' AAAGTCGATGTAGAGAGGAG 3'
PRR11-360-F 5' GAGAATTCTGCAGATATCCAG 3'

Primers used for RT-PCR analysis

PRR11	
F 5′-GACTTCCAAAGCTGTGCTTCC-3′	R5′-CTGCATGGGTCCATCCTTTTT-3′
Lamin B1	
5' –TGCCAAACTTGAGAATGCC-3'	5' –TGCGGCTTTCCATCAGTTC-3'
GAPDH	
5′-ACCTGACCTGCCGTCTAGAA-3′	5′-TCCACCACCCTGTTGCTGTA-3′
Topo IIa	
5' –AAACGGAATGACAAGCGAGAA- 3'	5' -CGTGGACTAGCAGAATCCTTG- 3'
CAP-E	

5' –ACACTGCGGGAACTTACAG- 3'	5' –GAAGCCCGAACCTGAGAC- 3'
CAP-G	
5' –CCAACCCTGCAAACACTGG- 3'	5' –TCTGGCGAGCACGGACTTG- 3'
CAP-D2	
5' –CCGCCAACTCTACCTCAACC- 3'	5'-CAAGACACTGAATGGTCCCAA- 3'

Supplementary table 2. Antibodies used in the present study

Primary antibody		IP	IF	IHC	WB
PRR11	Sigma-HPA023923		1:200		1:2000
Beta-actin	Abcam- Ab8226				1:5000
Lamin A/C	Abcam- Ab8984		1:500		1:2000
Lamin B1	Abcam- Ab16048		1:400		1:2000
Arp2	Santa cruz- sc-137250		1:50		
Arp2	Abcam- ab47654	1:50	1:500		1:2000
Arp3	Abcam- ab49671				1:1000
ARPC1A	Abcam- ab211124				1:1000
H3K9me3	Abcam- Ab8898		1:400		1:2000
Tubulin	Abcam- ab210797				1:1000
Caspase 3 (Asp175)	CST- 9661		1:200		
Histone H3.3	Abcam-ab176840				1:1000
Flag	Sigma-F7425		1:800		
Flag	Sigma-F1804	1:100			
phalloidin-TRITC	Sigma-P1951		1:1000		
Phalloidin-iFluor 488	Abcam- ab176753		1:200		
Secondary antibody					
Donkey anti-Mouse IgG (H+L) Alexa Fluor 488	Thermo Fisher ---A-21202		1:1000		
Donkey anti-Rabbit IgG (H+L) Alexa Fluor 488	Thermo Fisher ---A-21206		1:1000		
Donkey anti-Mouse IgG (H+L) Alexa Fluor 594	Thermo Fisher ---A-21203		1:1000		
Donkey anti-Rabbit IgG (H+L) Alexa Fluor 594	Thermo Fisher ---A-21207		1:1000		