Supplementary Information

Additional materials and methods

shRNA sequences are as follows:

Control sense

GATCCCCAACAGTCGCGTTTGCGACTGGTTCAAGAGACCAGTCGCAAACGCGAC
TGTTTTTTTGGAAA

Control antisense

AGCTTTTCCAAAAAAACAGTCGCGTTTGCGACTGGTCTCTTGAACCAGTCGCAA

ACGCGACTGTTGGG

shIKKβ sense

GATCCCCGAGGTGAGCAGATTGCCATTTCAAGAGAATGGCAATCTGCTCACCTGT
TTTTGGAAA

shIKKβ antisense

AGCTTTTCCAAAAAGAGGTGAGCAGATTGCCATTCTCTTGAAATGGCAATGTGCT
CACCTGGGG

siRNA sequences are as follows:

Control, CAGUCGCGUUUGCGACUGG (Anderson & Perkins, 2003)

IKKα, GCAGGCUCUUUCAGGGACA (van Uden et al, 2008)

IKKβ, CAGGUGAGCAGAUUGCCAU (van Uden et al, 2008)

IKKβ (B), AAUGCGGGAAUCUUAACGC

IKKβ (C), UGUCGUGCUAACUCUGUCG

IKKγ, ACAGGAGGUGAUCGAUAAG

RelA, GCUGAUGUGCACCGACAAG (Anderson & Perkins, 2003)

RelB, AAUUGGAGAUCAUCGACGAGU (van Uden et al, 2008)

c-Rel, AAAUGUGAAGGGCGAUCAGCA (van Uden et al, 2008)

p105/p50, AAGGGGCUAUAAUCCUGGACU (van Uden et al, 2008)

p100/p52, AAGAUGAAGAUUGAGCGGCCU (Schumm et al, 2006)

Claspin, UCAAUAAGAGCAGCUGUAA

Cyclin D1, UGUGUGCAGAAGGAGGUCTT (Schumm et al, 2006)

β-TrCP, GUGGAAUUUGUGGAACAUC (Mamely et al, 2006; Peschiaroli et al, 2006)

E2F1, CGCUAUGAGACCUCACUGA

Mouse IKKα, GCAGAAGAUUAUUGAUCUA

Mouse IKKβ, UGACGUGAAGCAUCUAGUA

Mouse IKKγ, GGAUUCGAGCAGUUAGUGA

PCR sequences are as follows:

β-actin:

For, GTGGGAGTGGGTGGAGGC

Rev, TCAACTGGTCTCAAGTCAGTG

IKKα

For, TGCACAAGTCATATTTAGGATGTG

Rev, GCAGAGAGGAGGACCTGTTG

IKKβ

For, CACTGCTTGATGGCAATCTG

Rev, AAATGAAAGAGCGCCTTGG

IKKγ

For, CTGGCTTGGAAATGCAGAAG

Rev, CTGCCTGGAGGAGAATCAAG

p100

For, AGCCTGGTAGACACGTACCG

Rev, CCGTACGCACTGTCTTCCTT

p105

For, GCACGACAACATCTCATTGG

Rev, TCCCAAGAGTCATCCAGGTC

RelA

For, CTGCCGGGATGGCTTCTAT

Rev, CCGCTTCTTCACACACTGGAT

RelB

For, TCCCAACCAGGATGTCTAGC

Rev, AGCCATGTCCCTTTTCCTCT

c-Rel

For, CTGCCTTCTTCAAGCTGGTC

Rev, CGCTTCCATTCCGACTATGT

Cyclin D1

For, GGCGGATTGGAAATGAACTT

Rev, TCCTCTCCAAAATGCCAGAG

Claspin

For, GAGTCAGAAGCCAGGTGGAG

Rev, TGCAGTGCTTTGGCTGTAAC

β–TrCP

For, CTAACCCTTCCCCTTCTTGG

Rev, GGTGAACCAGCTGAAAGCTC

E2F1

For-ATGTTTTCCTGTGCCCTGAG

Rev-ATCTGTGGTGAGGGATGAGG

Mouse Actin

For-ATGCTCCCCGGGCTGTAT

Rev-CATAGGAGTCCTTCTGACCCATTC

Mouse Claspin

For- TGCTGACGAAGAGGACATTG

Rev-CAGCAAGTTTTTGGAGCACA

ChIP Primers

Claspin Promoter Control Region

For, ACTTGGAAGAGGAGGCCAGT

Rev, CACCCTCCATGGTCATATCC

Claspin Promoter κB1

For, AAAAATTAGCCAGGGGTTGG

Rev, TGGAGTCTCCCTCTGTTAGCC

Claspin Promoter κB2

For, GCCTGGGTAATAGGAGATTGG

Rev, AGCAGTGCTGTTCTTGCTTTC

Claspin Promoter κB3

For, CCTCTTCAGCCGGTTCCT

Rev, CCCAGTGTGGAACTCCCTAA

IKK Kinase Assay

U2OS cells were treated with 10ng/ml TNF-α 5 minutes (min) prior to harvest to activate the IKK complex. To prepare WCLs, cells were washed with PBS, resuspended in cold kinase assay lysis buffer [20 mM Tris (pH 8.0), 500 mM NaCl, 1 mM EDTA, 1 mM EGTA, 10 mM NaF, 300 μM Na_3VO_4, 2 μM PMSF, 1x EDTA-free protease inhibitor

cocktail (Roche), 1 mM DTT, and 0.25% NP40], and lysed by sonication. Debris was removed by centrifugation, and extracts precleared with protein G-Sepharose beads for 1 hour (h) at 4°C. The IKK complexes were isolated by immunoprecipitation from a 500-µl reaction mixture of PD buffer [40 mM Tris (pH 8.0), 500 mM NaCl, 1 mM EDTA, 1 mM EGTA, 10 mM NaF, 300 µM Na_3VO_4, 2 µM PMSF, 1x EDTA-free protease inhibitor cocktail (Roche), 1 mM DTT, and 0.1% NP40) containing 500 µg of cytoplasmic proteins and 1 µg of antibody against either IKKα/β (H-470), or non-specific rabbit IgG. Complexes were captured using protein-G sepharose beads, and washed 3x with PD buffer. After washing, the immunoprecipitate was subjected to a kinase assay at 30°C for 45 min in kinase buffer C [20 mM HEPES (pH 7.7), 2 mM $MgCl_2$, 10 µM ATP, 10 mM NaF, 10 mM PNPP, 300 µM Na_3VO_4, 1 mM benzamidine, 2 µM PMSF, 1x EDTA-free protease inhibitor cocktail (Roche), and 1 mM DTT] containing 200 ng of either GST, wt GST-IκB-α fusion protein, or wt GST-Claspin (aa 1-64). The kinase reaction was stopped by the addition of 2x SDS-PAGE sample buffer, subjected to SDS-PAGE analysis, and visualized by immunoblotting using a specific phospho-serine antibody (Millipore). For radioactive kinase assay, kinase reaction was supplemented with ^{32}P-γ-ATP. The kinase reaction was stopped by the addition of 2x SDS-PAGE sample buffer, subjected to SDS-PAGE analysis, gel was dried and exposed to film.

RT-PCR

Semi-quantitative RT-PCR and PCR was performed as described before (Kenneth et al, 2009; Rocha et al, 2003). PCR products were resolved on 2% agarose gels and scanned using in a phosphorimager (FujiFilm FLA-5100) into TIFF format. Quantitative RT-PCR was performed using cDNA templates (cDNA synthesis was performed using Quantitect Reverse Transcription kit (Qiagen)) amplified using specific primer sets and the Stratagene Brilliant II

SYBR green qPCR mix according to the manufacturer instructions. Amplification and detection were performed using a Stratagene Mx3005P detection system. Sample values obtained with specific primer sets were normalized to β-actin primer set values.

Chromatin Immunoprecipitation (ChIP)

Proteins were cross-linked with formaldehyde for 10 min. 0.125 mM glycine was added, and cells washed with phosphate-buffered saline. Cells were lysed with lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCL, pH 8.1, 1 mM PMSF, 1 mg/ml leupeptin, 1 mg/ml aprotonin), followed by sonication and centrifugation. The supernatant was precleared with sheared salmon sperm DNA and protein G-Sepharose beads (Sigma). The supernatant was incubated with specific antibodies overnight, and then with protein G-Sepharose beads for 1 h. After an extensive wash step, the complexes were eluted with buffer (100 mM $NaHCO_3$, 1% SDS) and incubated with proteinase K. DNA was purified using a PCR purification kit (Qiagen or NBS). PCR was performed on the purified immunoprecipitated DNA using specific primers.

Antibodies

Antibodies used were: β-actin (A5441, Sigma), IKKα (2682, Cell Signaling), IKKβ (2678, Cell Signaling), IKKα/β (sc-7607, Santa Cruz Biotechnology), phospho IKKα/β (2681, Cell Signalling), IKKγ (sc-8330, Santa Cruz Biotechnology), phospho-IκBα serines 32/36 (9246, Cell Signaling), IκBα (4812, Cell Signaling), Ubiquitin (sc-8017, Santa Cruz Biotechnology), Cyclin D1 (2926. Cell Signaling), phospho-SMC1 serine 966 (A300-050A, Bethyl Laboratories), SMC1 antibodies (A300-055A, Bethyl Laboratories) phospho-Chk1 Ser 317 (2344, Cell Signaling), Chk1 (sc-8408, Santa Cruz Biotechnology), Polymerase II

CTD (sc-47701, Santa Cruz Biotechnology); RelB (sc-226, Santa Cruz Biotechnology), c-Rel (sc-71, Santa Cruz Biotechnology), p105/p50 (sc-7178, Santa Cruz Biotechnology), p100/p52 (sc-848, Santa Cruz Biotechnology; 05-361, Millipore), RelA (sc0372, Santa Cruz Biotechnology), Cyclin B1 (sc-752. Santa Cruz Biotechnology), Claspin (ab3720, Abcam; A300-266, Bethyl Laboratories; 2800 Cell Signaling), phospho-H2Ax (9718, Cell Signaling), Acetylated H3 (06-599, Millipore), anti-phospho-Serine (16-455, Millipore), anti-β-TrCP (373400, Invitrogen).

Cell Cycle Synchronisation

To arrest cells at the G1/S boundary, a double thymidine block and release experiment was performed where U2OS cells were treated for 16 h with 2.5 mM thymidine, washed twice in PBS, released into fresh media for 10 h, treated for a further 16 h with 2.5 mM thymidine, then released again into fresh media following two washes in PBS. To synchronise cells in mitosis, U2OS cells were treated for 16 h with 2.5 mM thymidine, washed 3x in PBS, and released into fresh media containing 100 ng/ml nocodazole (Sigma) for 18 h, cells were then washed 3x in PBS and released into fresh media.

Flow cytometric analysis of cell cycle distribution.

Cells were prepared for flow cytometry analysis as described (Schumm, 2006). Cells with DNA content between 2N and 4N were designated as being in the G1, S, or G2/M phase of the cell cycle. Cells with a DNA content < 2N were designated as being sub-G1 and cells >4N were designated as greater than G2. The number of cells in each compartment of the cell cycle was expressed as a percentage of the total number of cells present.

Assessment of DNA-checkpoint inactivation

U2OS cells were transfected with FLAG-tagged IKKβ SS-EE, or empty vector using GeneJuice transfection reagent. Cells were kept unsynchronized or trapped in prometaphase by incubating in the presence of nocodazole (100 ng/ml) for 12 h. Subsequently, cells were treated with UV (40 J/m^2) for 2 h, as indicated. DNA damage-dependent phosphorylation of Chk1 was analyzed by immunoblotting, using a phosphospecific antibody to Ser317 (Chk1-pS317).

Immunofluorescence and microscopy

For immunofluorescence, cells grown on coverslips and treated as indicated prior to fixation by incubation in 3.7% formaldehyde/PBS (pH 6.8) for 15 min. Cells were permeabilized in PBS-0.1% Triton X-100 for 15 min and then blocked in PBS-0.05% Tween supplemented with 1% normal donkey serum for 30 min. The dilutions of the antibodies used were: rabbit anti-phospho-H2Ax 1:100. Secondary antibody labelled with X-Red was purchased from Jackson Immunoresearch and used at 1:500. Cells were stained with primary antibodies without secondary antibodies to control for auto-florescence or stained with secondary antibodies alone to control for background staining. Cells were analysed and images were acquired using a DeltaVision microscope. Images were deconvoluted and analysed using OMERO client software (Open Microscopy Environment).

Additional References:

Anderson LA, Perkins ND (2003) Regulation of RelA (p65) function by the large subunit of replication factor C. *Mol Cell Biol* **23**(2)**:** 721-732

Kenneth NS, Mudie S, van Uden P, Rocha S (2009) SWI/SNF regulates the cellular response to hypoxia. *J Biol Chem* **284**(7)**:** 4123-4131

Mamely I, van Vugt MA, Smits VA, Semple JI, Lemmens B, Perrakis A, Medema RH, Freire R (2006) Polo-like kinase-1 controls proteasome-dependent degradation of Claspin during checkpoint recovery. *Curr Biol* **16**(19)**:** 1950-1955

Peschiaroli A, Dorrello NV, Guardavaccaro D, Venere M, Halazonetis T, Sherman NE, Pagano M (2006) SCFbetaTrCP-mediated degradation of Claspin regulates recovery from the DNA replication checkpoint response. *Mol Cell* **23**(3)**:** 319-329

Rocha S, Martin AM, Meek DW, Perkins ND (2003) p53 represses cyclin D1 transcription through down regulation of Bcl-3 and inducing increased association of the p52 NF-kappaB subunit with histone deacetylase 1. *Mol Cell Biol* **23**(13)**:** 4713-4727

Schumm K, Rocha S, Caamano J, Perkins ND (2006) Regulation of p53 tumour suppressor target gene expression by the p52 NF-kappaB subunit. *Embo J* **25**(20)**:** 4820-4832

van Uden P, Kenneth NS, Rocha S (2008) Regulation of hypoxia-inducible factor-1alpha by NF-kappaB. *Biochem J* **412**(3)**:** 477-484

Supplemental Figure Legends:

Sup. Figure 1.

The phospho-degron of Claspin is similar to that of IκBα. **(A)** Alignment of the amino acid regions corresponding to the phospho-degron motif in Claspin and IκBα. **(B)** Anti-IKKα/β immunoprecipitates from extracts of U2OS cells stimulated with TNF-α (10 ng/ml) for 5 min, were prepared for use in an immune complex kinase assay. Either 200 ng of GST, GST-IκB-α or GST-Claspin (1-64) was used as substrate in the presence of ^{32}P labelled γ-ATP. Reactions were stopped by addition of 2x SDS loading buffer, and analysed by western blotting using the indicated antibodies or subjected to SDS-PAGE analysis, and visualized by autoradiography.

Sup. Figure 2

IKKβ controls Claspin levels in different cell lines. (A) Quantitative RT-PCR analysis of IKKα and IKKβ mRNA confirming the knockdown in cells prepared from U2OS cells depleted of each of the IKK subunits respectively. **(B)** U2OS cells were depleted of IKKβ using multiple siRNAs against IKKβ. WCLs were prepared and analysed by western blot using the indicated antibodies **(C)** U2OS cells were co-transfected with a non-targeting siRNA or two distinct siRNAs to deplete IKKβ, along with either a vector control or a Flag-tagged version of IKKβ. WCLs were prepared and analysed by western blot using the indicated antibodies. **(D)** MDA-MB-231 or HEK 293 cells were depleted of the indicated IKK subunits using siRNA. **(E)** U2OS cells were co-transfected with a NF-κB luciferase reporter and siRNA oligonucleotides as indicated for 48 h prior to lysis and luciferase activity measured. Graph depicts relative luciferase activity compared to control siRNA transfected

cells. Anova student t-test were performed on the means and levels of significance are indicated as follows: *<0.050, **<0.010, ***<0.001.

Sup. Figure 3

IKKβ controls Claspin levels in MEFs. (A) Mouse Embryonic Fibroblasts (MEFs) were treated, as indicated with the IKK inhibitor, BAY- 11-7082, for 24 h. WCLs were subjected to immunoblot analysis for the levels of the indicated proteins. **(B)** MEFs were depleted of IKKα, IKKβ or IKKγ using specific siRNAs and WCLs were prepared and analysed by western blot using the indicated antibodies. **(C)** Quantitative RT-PCR analysis of Claspin mRNA confirming the knockdown in cells prepared from MEFs depleted of IKKα, IKKβ or IKKγ using specific siRNAs. Anova student t-test were performed on the means and levels of significance are indicated as follows: *<0.050, **<0.010, ***<0.001.

Sup Figure 4

IKKβ controls Claspin mRNA levels. (A) Quantitative RT-PCR analysis of Claspin mRNA prepared from MDA-MB-231 cells depleted of IKKβ using siRNA. **(B)** Quantitative RT-PCR analysis of Claspin mRNA prepared from U2OS cells depleted of IKKβ using alternative siRNAs. Anova student t-test were performed on the means and levels of significance are indicated as follows: *<0.050, **<0.010, ***<0.001.

Sup. Figure 5

RNAi against the different NF-κB subunits significantly depletes their mRNA. Quantitative RT-PCR analysis of RelA, RelB, c-Rel, p105/ p50 and p100/ p52 mRNA confirming the knockdown in cells prepared from U2OS cells depleted of each of the NF-κB subunits respectively. Anova student t-test were performed on the means and levels of significance are indicated as follows: *<0.050, **<0.010, ***<0.001.

Sup. Figure 6

Characterisation of shRNA IKKβ U2OS cells. U2OS cells were stably depleted of IKKβ using shRNA. WCLs were subjected to immunoblot analysis for the levels of the indicated proteins.

Sup. Figure 7

IKK-NF-κB protein levels are unchanged during the cell cycle. (A) U2OS cells were depleted of Cyclin D1 with siRNA prior to fixation and FACs analysis. Results are depicted as the percentage of each cell population in each phase of the cell cycle. **(B)** U2OS cells were synchronised at G1/S using a double thymidine block. Thymidine was subsequently washed and cells were released for the indicated times. Cells were analysed by flow cytometry and % of cells in each stage of the cell cycle is indicated. WCLs were prepared from these cells and analysed by western blot using the indicated antibodies. **(C)** U2OS cells were synchronised at prometaphase using nocodazole. Nocodazole was subsequently washed and cells were analysed by flow cytometry and % of cells in each stage of the cell cycle is indicated. WCLs were prepared and analysed by western blot using the indicated antibodies.

Sup. Figure 8

RNAi against β-TrCP does not alter basal c-Rel/ IKK activity. (A) U2OS cells were depleted of β-TrCP using siRNA. WCLs were subjected to immunoblot analysis for the levels of the indicated proteins. Quantitative RT-PCR analysis of β-TrCP mRNA prepared from U2OS cells depleted of β-TrCP using siRNA. Anova student t-test were performed on the means and levels of significance are indicated as follows: *<0.050, **<0.010, ***<0.001. **(B)** U2OS cells depleted of β-TrCP using siRNA were compared to cells stimulated with 10ng/ml TNF-α for 10 min. WCLs were prepared and analysed by western blotting using the indicated antibodies. **(C)** Nuclear and cytoplasmic extracts were prepared from control cells or cells depleted of β-TrCP. WCLs were prepared and analysed by western blotting using the indicated antibodies.

Sup. Figure 9

PMA/ Ionomycin induces Clapsin in an IKK dependent manner. (A) Quantitation of the increase in Claspin protein levels in U2OS treated with PMA (100 ng/mL)/Ionomycin (0.5 μM) for 4 h. **(B)** U2OS cells were depleted of IKKβ using siRNA. Cells were treated with PMA (100 ng/mL)/Ionomycin (0.5 μM) for 4 h prior to harvest as indicated. WCLs were prepared and analysed by western blot using the indicated antibodies.

Sup. Figure 10

IKKβ controls Chk1 phosphorylation in different cell lines. (A) MDA-MB-231 cells were depleted of IKKβ with siRNA. Cells were treated with UV (40 J/m^2) as indicated, and

harvested 4 h later. WCLs were prepared and analysed by western blot using the indicated antibodies. **(B)** U2OS cells were depleted of IKKβ using siRNA. Cells were then treated with 3mM hydroxyurea and harvested 4 h later. WCLs were prepared and analysed by western blotting using the indicated antibodies. **(C)** U2OS cells were depleted of IKKβ with siRNA. Cells were treated with UV (40 J/m^2) as indicated, and harvested 4h later. WCLs were prepared and analysed by western blot using the indicated antibodies. **(D)** HEK293 cells were transfected with vector control or constitutively active IKKβ prior to treatment with UV (40 J/m^2) as indicated, and harvested 4 h later. WCLs were prepared and analysed by western blot using the indicated antibodies.

Sup. Figure 11

IKK and c-Rel depletion alter Claspin function. (A) U2OS cells were depleted of the indicated proteins using RNAi. 48 h later cells were fixed and stained with anti-phospho-H2Ax antibody and DAPI. Cells were visualised by immunofluorescence using a DeltaVision microscope. Cells presenting H2Ax foci were counted, and percentage of cells calculated. Graph depicts mean percentage values plus standard deviation of a total of 500 cells counted for each condition. Anova student t-tests were performed on the means. *** $p<0.001$. **(B)** U2OS cells stably depleted of IKKβ were treated with UV (40 J/m^2) for 24 and 48 h prior to fixation and FACs analysis. Results are depicted as the percentage of each cell population in each phase of the cell cycle.

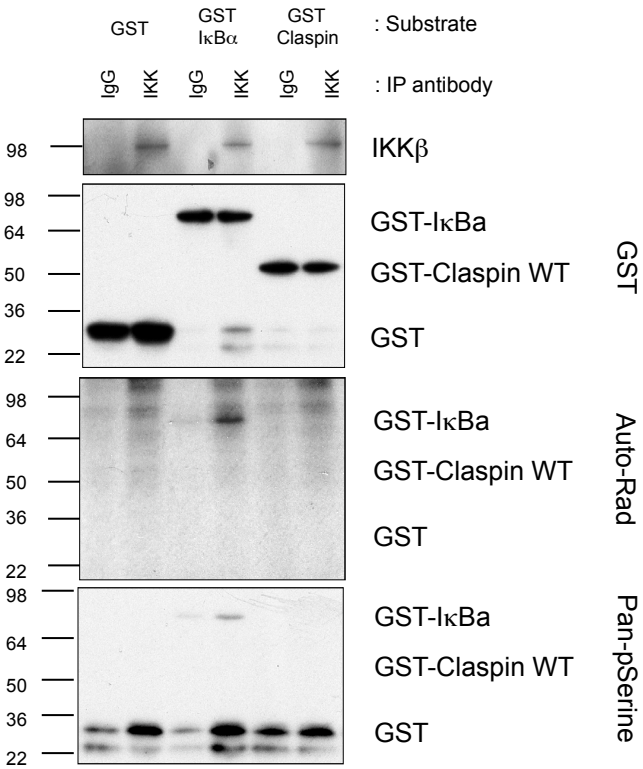
Sup. Figure 12

RNAi against E2F1 does not significantly alter Claspin mRNA. Quantitative RT-PCR analysis of Claspin, E2F1, and IKKβ mRNA, following knockdown of E2F1 and IKKβ in U2OS cells using specific siRNAs. Anova student t-test were performed on the means and levels of significance are indicated as follows: *<0.050, **<0.010, ***<0.001.

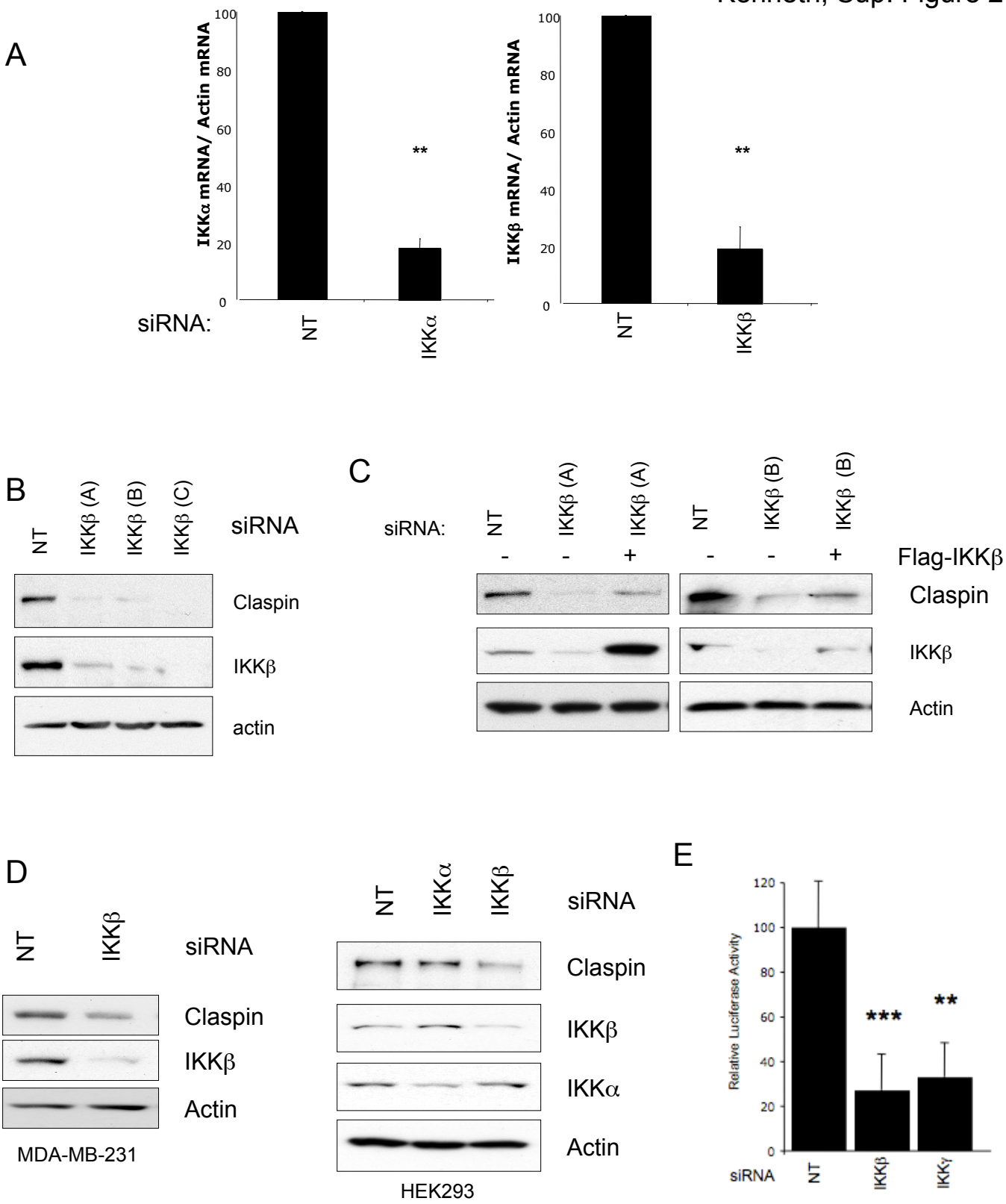
A

Claspin (Human)26 – SPS**DSG**QG**S**YET – 37
Claspin (Mouse)26 – SPV**DSG**QG**S**FET – 37
IκBα (Human)28 – DRH**DSG**LD**S**MKD - 39

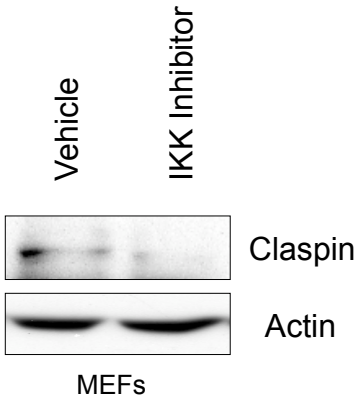
B



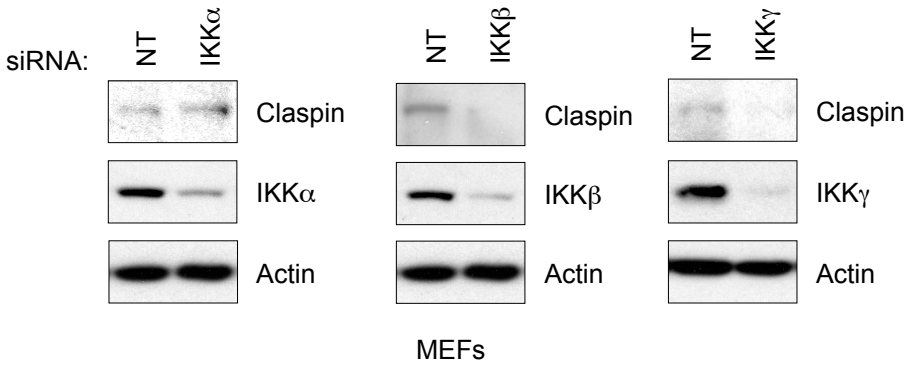
Kenneth, Sup. Figure 2



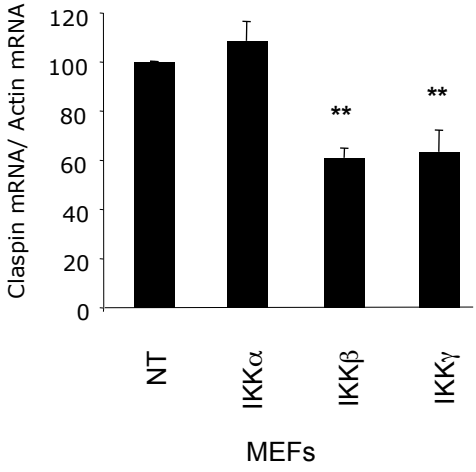
A


Vehicle
IKK Inhibitor
Claspin
Actin
MEFs

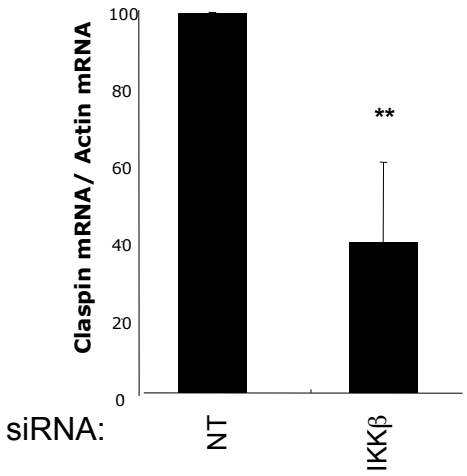
B


siRNA:
NT
IKKα
NT
IKKβ
NT
IKKγ
Claspin
IKKα
Actin
Claspin
IKKβ
Actin
Claspin
IKKγ
Actin
MEFs

C

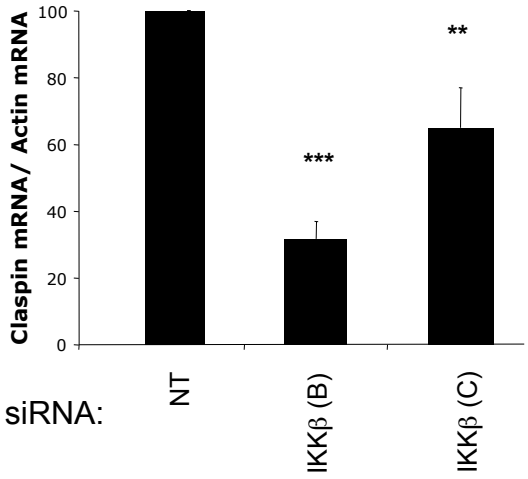

Claspin mRNA/ Actin mRNA
120
100
80
60
40
20
0
**
**
NT
IKKα
IKKβ
IKKγ
MEFs

A

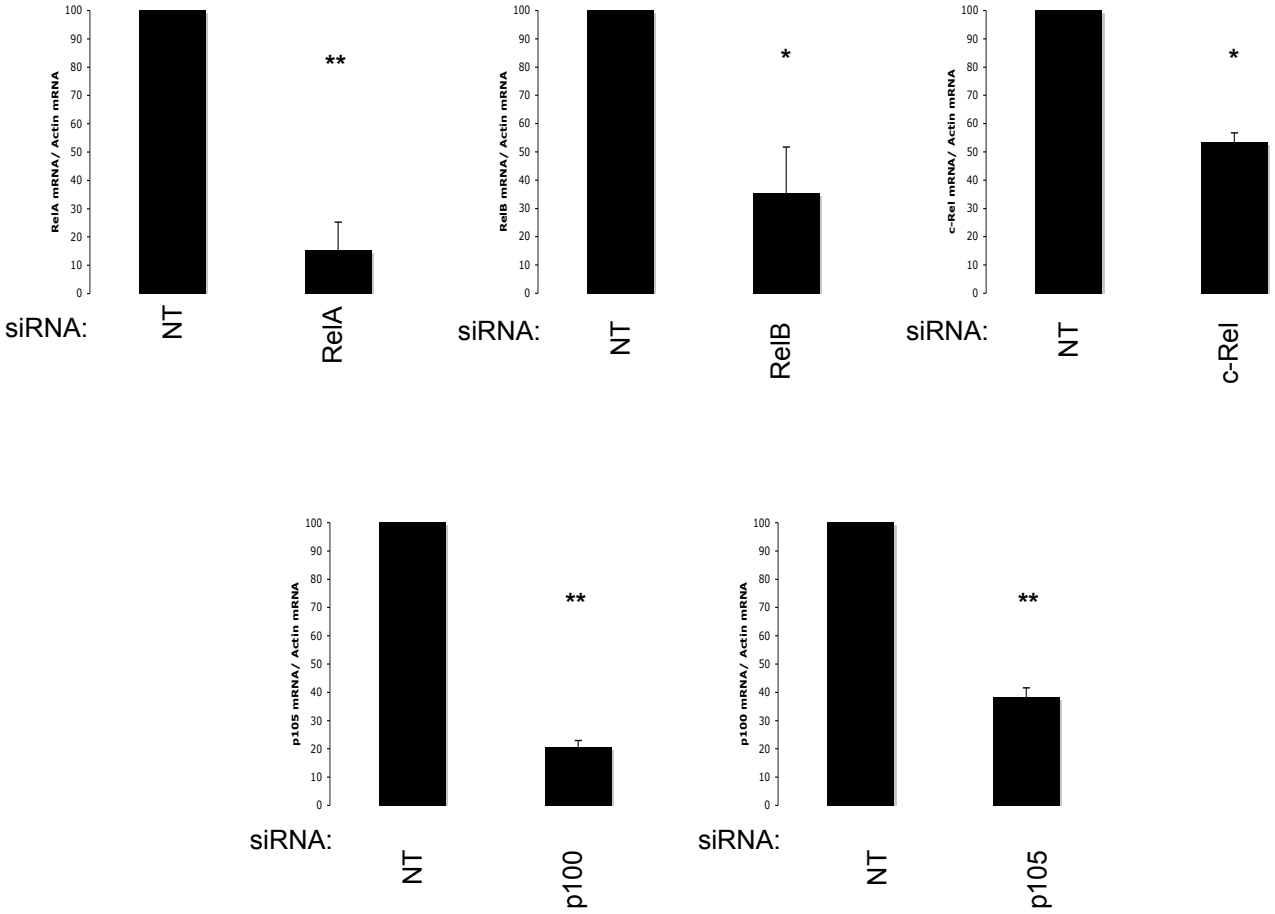


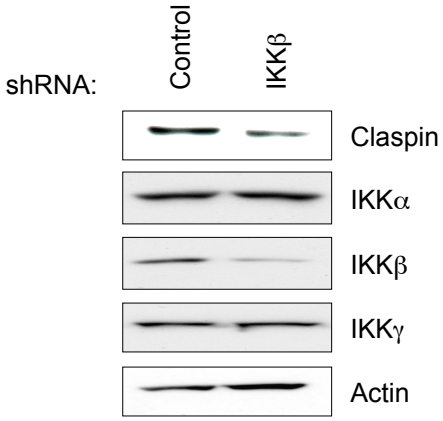
MDA-MB-231

B

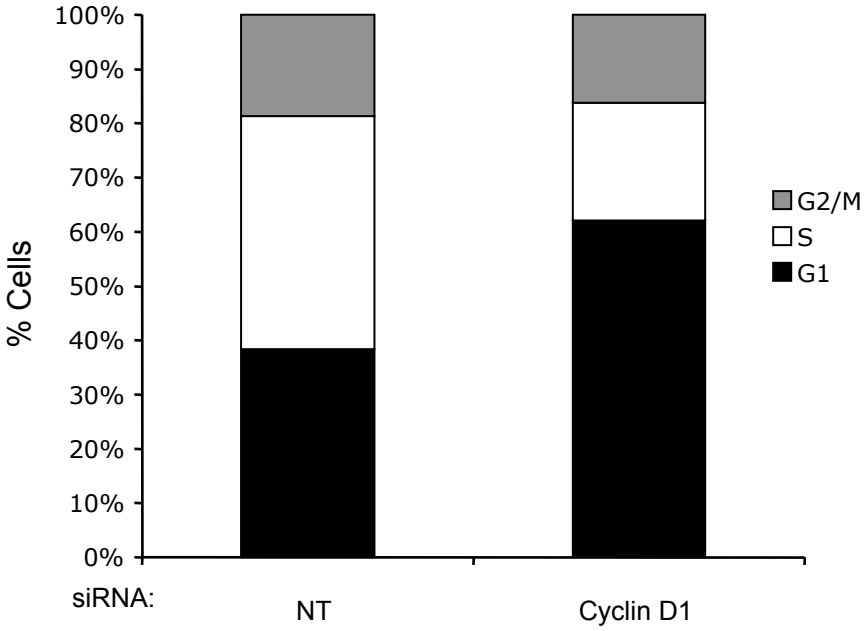


U2OS

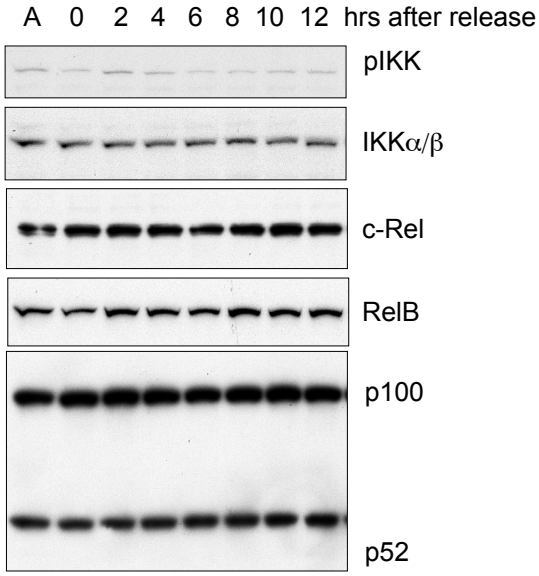

RelA mRNA/ Actin mRNA
100
90
80
70
60
50
40
30
20
10
0
**
siRNA:
NT
RelA
RelB mRNA/ Actin mRNA
*
siRNA:
NT
RelB
c-Rel mRNA/ Actin mRNA
*
siRNA:
NT
c-Rel
p105 mRNA/ Actin mRNA
**
siRNA:
NT
p100
p100 mRNA/ Actin mRNA
**
siRNA:
NT
p105


shRNA:
Control
IKKβ
Claspin
IKKα
IKKβ
IKKγ
Actin

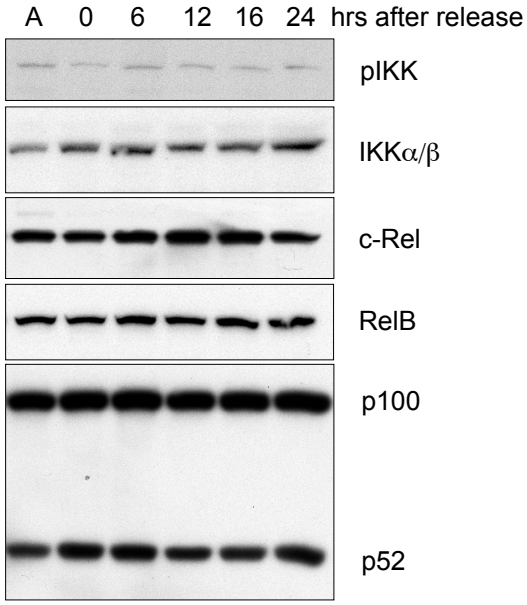
A

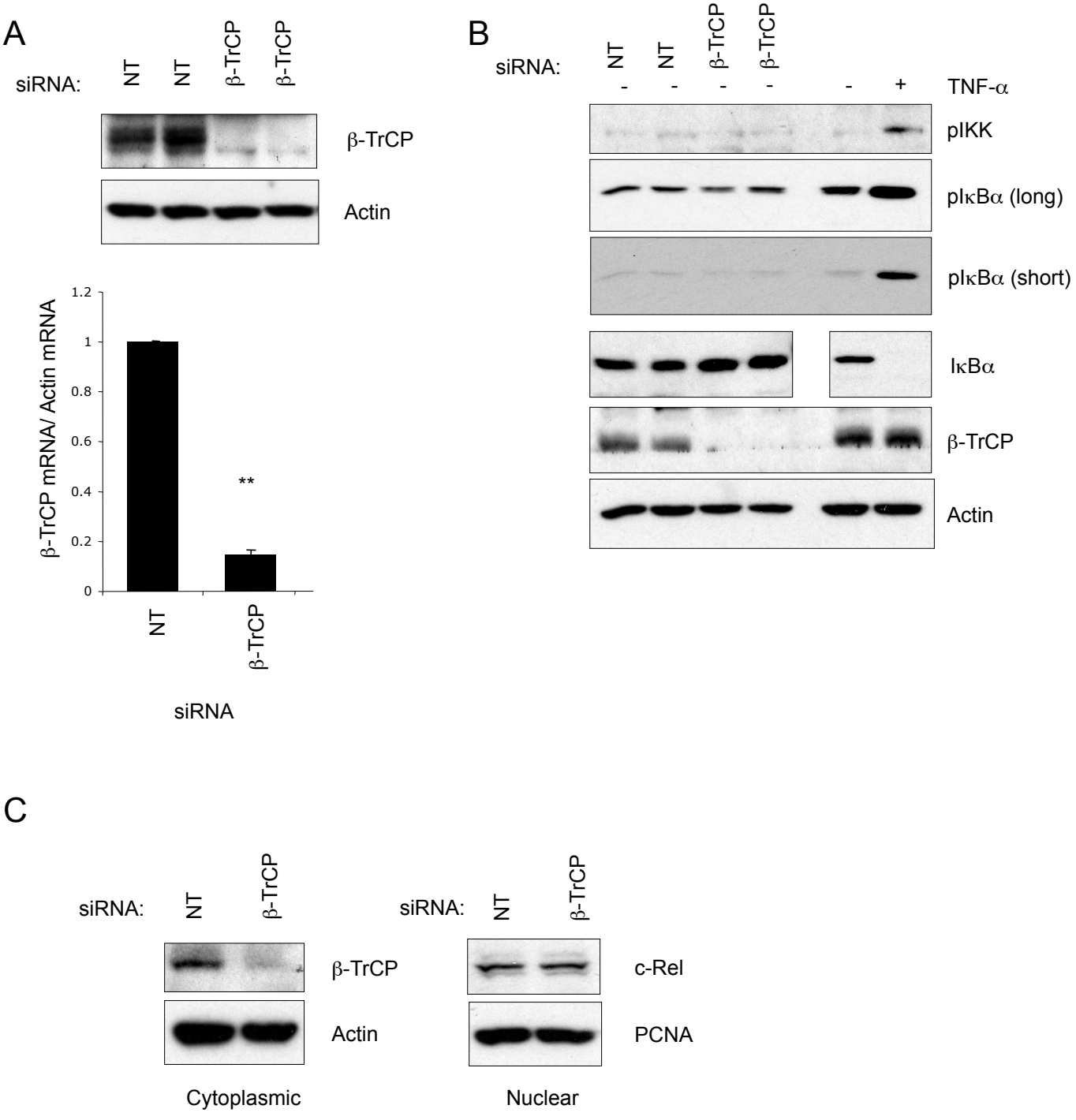

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% Cells
G2/M
S
G1
siRNA:
NT
Cyclin D1

B

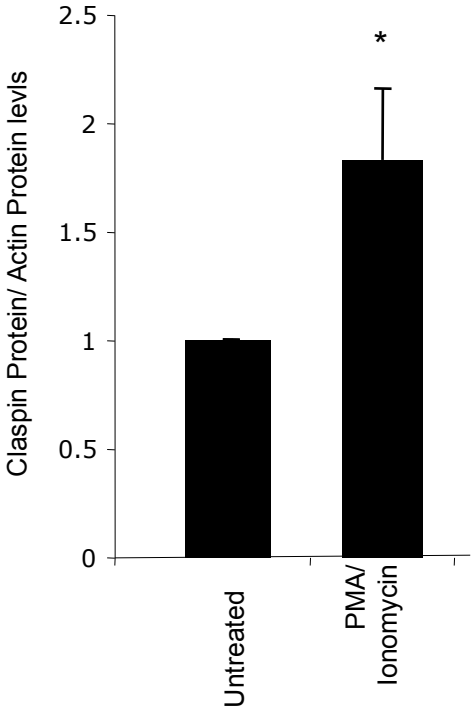

A 0 2 4 6 8 10 12 hrs after release
pIKK
IKKα/β
c-Rel
RelB
p100
p52

C

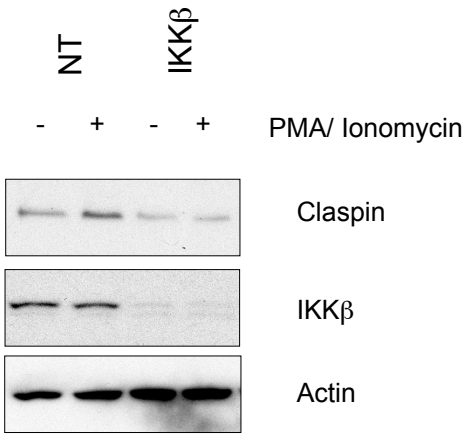

A 0 6 12 16 24 hrs after release
pIKK
IKKα/β
c-Rel
RelB
p100
p52


A
siRNA:
NT
NT
β-TrCP
β-TrCP
β-TrCP
Actin
β-TrCP mRNA/ Actin mRNA
1.2
1
0.8
0.6
0.4
0.2
0
**
NT
β-TrCP
siRNA
B
siRNA:
NT
NT
β-TrCP
β-TrCP
-
-
-
-
-
+
TNF-α
pIKK
pIκBα (long)
pIκBα (short)
IκBα
β-TrCP
Actin
C
siRNA:
NT
β-TrCP
β-TrCP
Actin
Cytoplasmic
siRNA:
NT
β-TrCP
c-Rel
PCNA
Nuclear

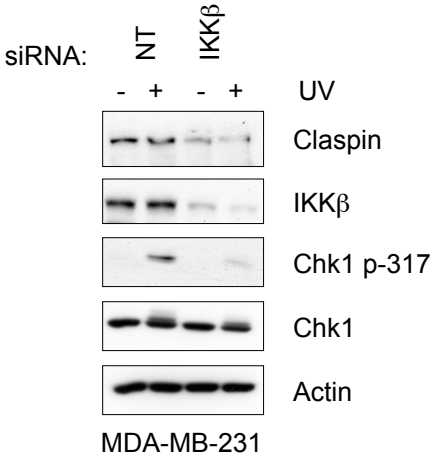
A


Claspin Protein/ Actin Protein levls
2.5
2
1.5
1
0.5
0
*
Untreated
PMA/
Ionomycin

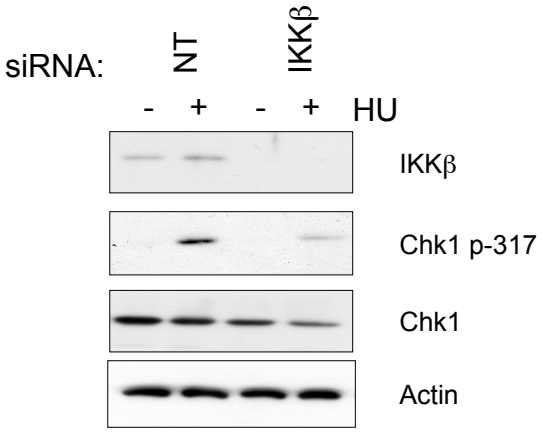
B


NT
IKKβ
- + - +
PMA/ Ionomycin
Claspin
IKKβ
Actin

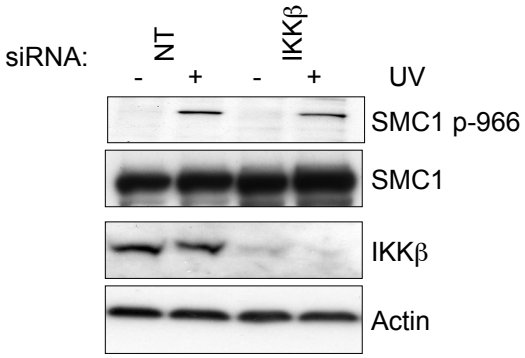
A


siRNA:
NT
IKKβ
- + - +
UV
Claspin
IKKβ
Chk1 p-317
Chk1
Actin
MDA-MB-231

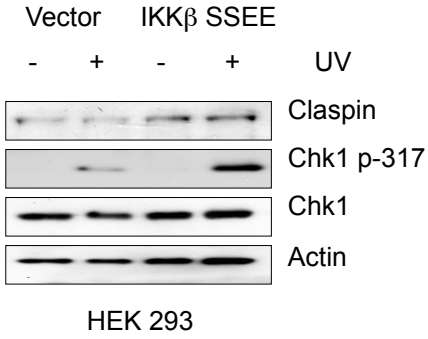
B


siRNA:
NT
IKKβ
- + - +
HU
IKKβ
Chk1 p-317
Chk1
Actin

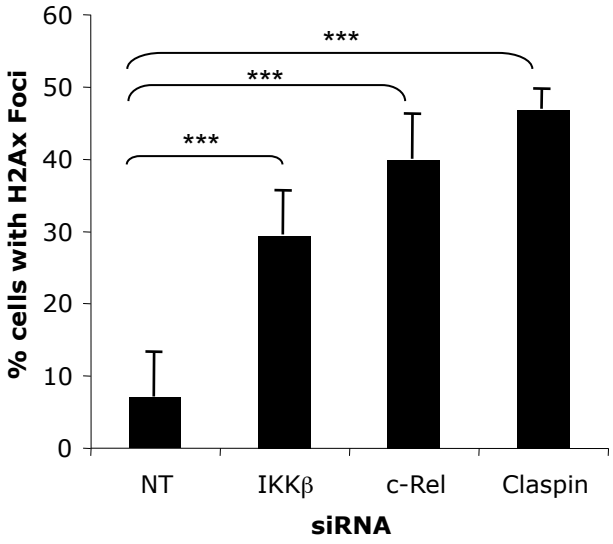
C


siRNA:
NT
IKKβ
- + - +
UV
SMC1 p-966
SMC1
IKKβ
Actin

D

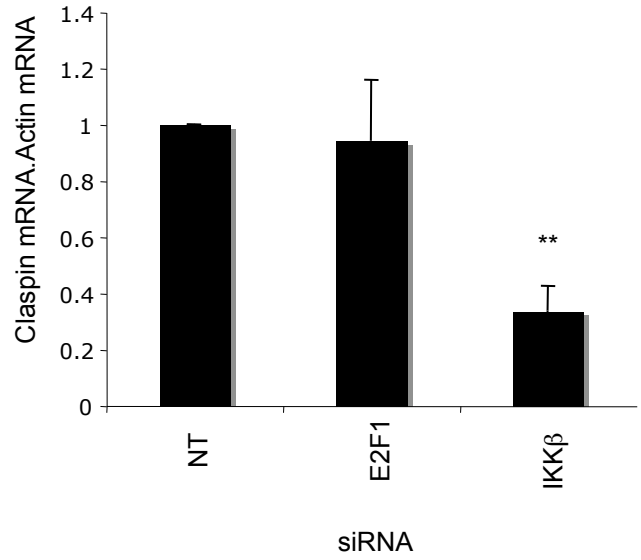

Vector
IKKβ SSEE
- + - +
UV
Claspin
Chk1 p-317
Chk1
Actin
HEK 293

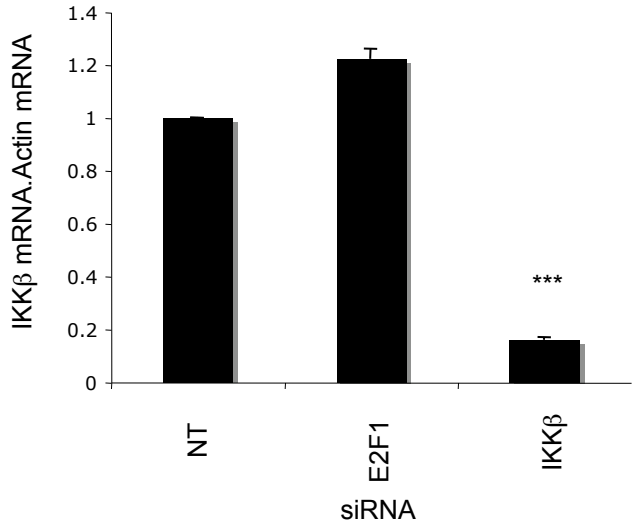
A

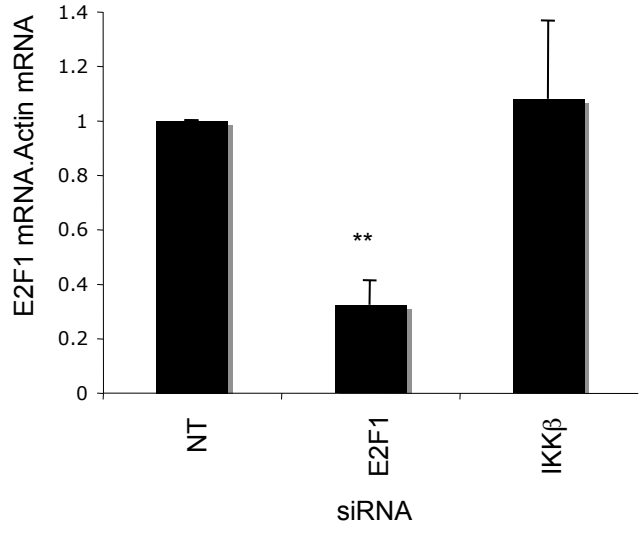


B

shRNA	UV	% Sub G1	%G1	%S	%G2/M	%>G2
NT	0hr	0.96	45.62	27.89	23.18	2.26
NT	24hr	2.99	46.76	27.95	21.75	1.45
NT	48hr	9.02	46.29	22.18	21.62	1.53
shIKKβ	0hr	1.42	51.91	19.88	23.82	2.79
shIKKβ	24hr	4.28	47.60	21.75	25.89	1.45
shIKKβ	48hr	24.08	35.77	18.11	20.65	2.12


Claspin mRNA.Actin mRNA
1.4
1.2
1
0.8
0.6
0.4
0.2
0
**
NT
E2F1
IKKβ
siRNA


IKKβ mRNA.Actin mRNA
1.4
1.2
1
0.8
0.6
0.4
0.2
0
***
NT
E2F1
IKKβ
siRNA


E2F1 mRNA.Actin mRNA
1.4
1.2
1
0.8
0.6
0.4
0.2
0
**
NT
E2F1
IKKβ
siRNA