Appendix Materials for

The IκB kinase complex is a regulator of mRNA stability

Mikuda, N.*, Kolesnichenko, M.*, Beaudette, P., Popp, O., Uyar, B., Sun, W., Tufan, A.B., Perder, B., Akalin, A., Chen, W., Mertins, P., Dittmar, G., Hinz, M., and Scheidereit, C.**

* equal first authors
** corresponding author, E-mail: scheidereit@mdc-berlin.de

This file includes:

- Legends to Appendix Figs. S1 to S8
- List of Tables EV1-EV8
- Appendix References
- Appendix Figs. S1 to S8

LEGENDS TO SUPPLEMENTARY FIGURES

Figure S1. Genotoxic stress augments EDC4 interaction with IKKγ.

A) Heatmap of proteins with enhanced binding to IKKγ after IR-treatment. U2-OS cells were labelled with heavy and light amino acids. For the forward experiment (fwd), heavy labelled cells were irradiated and harvested 45 min later. Light labelled cells were left untreated. For the reverse experiment, labels were switched. Cytoplasmic extracts (CE) were prepared and IKKγ and its interaction partners were immunoprecipitated with an anti-IKKγ antibody crosslinked to Dynabeads. IP samples were subjected to MS analysis. Cut-off for increased interaction in irradiated compared to unstimulated cells was set for at least 2-fold change, resulting values for the H/L ratio of at least 2 in the fwd experiment and 0.5 in the reverse experiment (Table EV1). Note that the time point taken after irradiation is later than time frame in which the IKKγ-PARP-1-PIASy-ATM interactions can be observed(Stilmann et al, 2009) (and data not shown). Identified targets were scored using two repositories annotating the frequency of detection in co-immunoprecipitation experiments or in negative controls from large scale MS experiments, respectively (http://www.peptracker.com/pfl and www.crapome.org). Targets were screened for predicted IKK phosphorylation sites using the dbPTM database (http://dbptm.mbc.nctu.edu.tw/). IKKγ interacting proteins with documented function in mRNA metabolism are highlighted in yellow. **B)** GO term clusters of interaction partners of IKKγ identified in **A)**. **C)** Immunoprecipitation of endogenous IKKβ or EDC4 with and without RNase-treatment prior to IP (RNaseA /T1 cocktail mix) from unstimulated (ut), irradiated (20 Gy; 45 min post stimulus) or TNFα-treated (10 ng/ml; 15 min) U2-OS cells. WB of EDC4 or IKKβ. **D)** HEK293 cells bearing a doxycycline-inducible HA-FLAG-tagged DDX6 plasmid were left untreated or pre-treated with dox for 3 days. Dox-treated cells were left unstimulated or were irradiated with 20 Gy and harvested at indicated time points. DDX6 was immunoprecipitated by pull-down of the HA-tag followed by SDS-PAGE western blot of FLAG (DDX6), and IKKγ. **E)** U2-OS cells were irradiated (IR; 20 Gy, 45 min) or left unstimulated (ut) and cytoplasmic (CE) and nuclear (NE) extracts were prepared. Cytoplasmic lysates were used for immunoprecipitation of TRAF6 followed by SDS-PAGE western blot of TRAF6, IKKγ, and EDC4. **F)** *In vitro* co-immunoprecipitation followed by SDS-PAGE western blot of purified recombinant Strep-tagged EDC4 sub-domains with recombinant GST-tagged full-length IKKγ by immunoprecipitation with anti-GST-beads. **G)** EDC4 diagram with WD40 domain, serine-rich linker and α-helical domain, including summary of interaction of IKKγ with EDC4 or EDC4 domains shown in Fig. 1E and Fig. S1F. **H)** IKKγ diagram with coiled-coil domains 1 and 2 (CC1, CC2), leucine zipper (LZ) and zinc finger (ZNF) and summary of EDC4-WD40 domain interactions (see Fig. 1F-G).

Figure S2. IKK phosphorylates EDC4 at S583 in cells

Endogenous EDC4 purified from unstimulated or TNFα-treated (10 ng/ml, 15 min) U2-OS CRISPR cells (15 cm dish per IP) (wildtype control, WT; IKKβ knockout, IKK-KO; EDC4 knockout; EDC-KO) by immunoprecipitation of EDC4 and subsequently analysed for phosphorylated peptides by LC-MS/MS analysis. **A)** MS2 spectrum of a phosphopeptide, showing endogenous EDC4-phosphorylation on Ser583 from a TNFα-treated sample. Figure exported from MaxQuant Viewer. Coverage of b- and y ions indicated by blue and red colors, respectively (see Table EV3 for MS data). **B)** SDS-PAGE/ western blot probed with antibodies against indicated proteins from input of samples used for MS-IP (see a). **C)** Phospho-site intensities of EDC4 Ser583-P [IVELPAPADFLS(P)LSSETKPK] normalized to overall EDC4 unmodified protein intensities. Intensities reported are precursor signals from the respective phosphopeptides. n.d.: not detectable. **D)** Reported phosphosites of EDC4 (see phosphositeplus.org). Phosphosites of EDC4 identified in this study are indicated.

Figure S3. Phosphorylation of EDC4 by IKK promotes P-body formation.

A) P-body foci visualized by fluorescence microscopy using anti-DDX6 antibody (red) and EDC4 (green) in primary BJ cells left untreated (ut), at 45 minutes (45') or 90 minutes (90') post irradiation (IR = 80 Gy). Nuclei were stained with DAPI (blue). **B)** Visualization of P-body foci in HeLa cells as in **A)** (IR = 10 Gy). **C)** Fluorescence microscopy using anti-EDC4 antibody (red) and anti-IKKβ antibody (green) in U2-OS cells as in Figure 3 A). **D)** Fluorescence microscopy using anti-EDC4 antibody (red) and anti-IKKγ antibody (green) in U2-OS cells as in Figure 3 A). **E-G)** SDS-PAGE/ western blot probed with antibodies against indicated proteins from whole cell lysates of unstimulated U2-OS cells, or cells treated with TNFα (10 ng/ml), IL-1β (10 ng/ml), or H_2O_2 (100 μM) for the indicated times. **H-J)** Visualization of P-body foci of U2-OS cells treated with TNFα (**H)**, IL-1β (**I)** and H_2O_2 (**J)**. Cells were stained with antibody against DDX6 (green). Nuclei were visualized with DAPI (blue). **K)** Left: SDS-PAGE/ western blot of clonal U2-OS cells stably expressing pTRIPZ RFP-coupled shIKKβ. To induce depletion of IKKβ, cells were treated with doxycycline (IKK^{sh}) or left untreated (wt). Cells were harvested 45 or 90 minutes post irradiation. Right, top panel: EMSA of nuclear extracts from U2-OS cells stably expressing pTRIPZ RFP-coupled shIKKβ treated with doxycycline (IKK^{sh}) or left untreated (wt). Cell extracts were prepared 45 or 90 min post irradiation. Lower panel: SDS-PAGE blot probed for PARP-1 as loading control. **L)** P-bodies visualized by fluorescence microscopy using anti-DDX6 antibody (green) and nuclei stained with DAPI (blue) in clonal U2-OS cells stably expressing pTRIPZ RFP-coupled shIKKγ. To induce depletion of IKKγ, cells were treated with doxycycline ($IKK\gamma^{sh}$) or left untreated (IKKγ wt). Cells were analysed 45 or 90 min post irradiation. **M)** Top panel: SDS-PAGE

of clonal U2-OS cells stably expressing pTRIPZ RFP-coupled shIKKγ, as in **K).** Lower panel: EMSA of nuclear extracts from clonal U2-OS cells stably expressing pTRIPZ RFP-coupled shIKKγ, as in **K)**. **N)** P-bodies visualized by fluorescence microscopy using anti-DDX6 antibody (green) in U2-OS cells transfected with control siRNA (scr_si) or siRNA directed against IKKγ (IKKγ_si). Analysed cells were untreated or 45 min post irradiation. Nuclei were stained with DAPI (blue). **O)** U2-OS cells were pre-treated with DMSO or IKK inhibitor (BMS345541) and analysed by fluorescence microscopy. Cells were stained with antibodies against p65 (red), DDX6 (green) and nuclei were stained with DAPI (blue). Analysis was performed with untreated cells or 45 min and 90 min post irradiation. Right panel: SDS-PAGE blot of whole cell extracts from U2-OS cells pre-treated with DMSO or IKK inhibitor and analysed 45 min after irradiation. **P)** Visualization of P-bodies in clonal U2-OS cells stably expressing pTRIPZ RFP-coupled shEDC4 as in **L)**. **Q)** SDS-PAGE of clonal U2-OS cells stably expressing pTRIPZ RFP-coupled shEDC4, as in **M)**. **R)** U2-OS cells were transfected with siRNA against EDC4 (EDC4_si) or control siRNA (scr_si) and treated as in **N)**. **S)** Visualization of P-bodies in CRISPRV2 ctrl (no guide RNA), EDC4 or IKKβ U2-OS knockout cells. Cells were left unstimulated (ut), or were irradiated (20 Gy; analysis 45 min post stimulus) or treated with TNFα (10 ng/ml; analysis 45 min post stimulus). Left panel: Cells were stained with antibody against DDX6 (green) and EDC4 (red). Nuclei were visualized with DAPI (blue), scale bar: 20 µm. Right panel: Quantification of P-body foci (blind count) from independent experiments (n=3) by ImageJ software, 500 cells per experiment ± s.d. unpaired t-test, *P < 0.05.

Figure S4. Significant formation of stress granules is only induced by hydrogen peroxide.
A) Visualization of P-body foci and of stress granules in U2-OS cells treated with IR (20 Gy), TNFα (10 ng/ml), IL-1β (10 ng/ml), or H_2O_2 (100 µM) for indicated times by fluorescence microscopy. P-body foci were visualized using anti-EDC4 antibody (red), formation of stress granules was analysed using anti-G3BP1 antibody (green). Nuclei were stained with DAPI (blue). **B)** Enlarged sections of immunofluorescence images from cells 180 min post stimulus from **A)**. White arrow: P-body; red arrow: stress granule.

Figure S5. EDC4 and IKK regulate basal and DNA damage-induced stability of multiple transcripts.

A) Graph illustrating mRNAs showing increased (green) or decreased (red) expression in irradiated U2-OS cells (20 Gy, 1h) relative to unstimulated cells. mRNA expression was measured by RNA-Seq, significance was calculated for two corresponding conditions and cut-off set for p-value < 0.1 and fold change > 2 (Table EV5A) **B)** Graph illustrating mRNAs showing increased (green) or decreased (red) stability in irradiated cells relative to unstimulated cells. mRNA expression was measured by RNA-Seq, significance was calculated for 2 corresponding conditions and cut-off set for p-value < 0.1 and fold change > 1.5 (Table EV5B). **C)** Visualization of GO terms of transcripts showing altered expression in irradiated cells relative to unstimulated cells (as in Figure 4 F) (Table EV4D). **D)** Visualization of GO terms of transcripts showing altered stability in irradiated cells relative to unstimulated cells using REVIGO (as in Fig. 4F) (Table EV5C). **E)** Visualization of GO terms of transcripts showing increased expression in IKK- or EDC4-depleted cells using REVIGO (Supek et al, 2011) (as in Fig. 4F) (Table EV4G). **F)** Visualization of GO terms of transcripts showing decreased expression in IKK- or EDC4-depleted cells (as in Fig. 4F) (Table EV4H).

Figure S6. Phosphorylation of EDC4 by IKK regulates stability of IL-8 mRNA. A) Analysis of stability of IL-8 mRNA by actinomycin chase assay. U2-OS cells were left untreated or irradiated (20 Gy). 30 or 60 min post-IR, cells were treated with actinomycin D (ActD; 10 mg/ml) to inhibit transcription and harvested at indicated time points. Residual mRNA expression following ActD treatment was assessed by qRT-PCR. Left: Relative normalized expression is calculated with reference to two controls (Rpl13a and HPRT1) and untreated, unstimulated control is set to 1. Right: Residual mRNA expression after actinomycin D treatment given as percentage of respective untreated sample. Quantification is representative for independent experiments (n>3); ± s.d. unpaired t-test, *P < 0.01. **B)** qRT-PCR analysis of IL-8 exon-exon (mature mRNA) (top left panel) and IL-8 exon-intron (nascent pre-mRNA) (top right panel) RNA from U2-OS cells stimulated by IR, and 60 min later treated with actinomycin D for 120 min (ActD; 10 mg/ml) to inhibit transcription. Relative normalized expression is calculated with reference to two controls (Rpl13a and HPRT1) and untreated, unstimulated control is set to 1. cDNA was transcribed in presence of reverse transcriptase (+RT) or without addition of reverse transcriptase (-RT). Quantification is representative for independent experiments (n>3); ± s.d. unpaired t-test, *P < 0.01. **C)** Quantitative RT-PCR analysis of IL-8 mRNA from primary BJ cells stimulated by IR (80 Gy), and after 60 min treated with actinomycin D for 0, 60 and 120 min (ActD; 10 mg/ml). Relative normalized expression is calculated with reference to two controls (Rpl13a and HPRT1) and untreated, unstimulated control is set to 1. Quantification is representative for independent

experiments (n>3); ± s.d. unpaired t-test, *P < 0.01. **D)** qRT-PCR analysis of IL-8 mRNA from HepG2 cells treated as in **C)** (IR = 40 Gy)**. E)** qRT-PCR analysis of IL-8 mRNA from HeLa cells treated as in B) (IR = 10 Gy)**. F)** Left panel: qRT-PCR analysis of IL-8 mRNA from U2-OS cells stimulated for 30 or 60 with TNFα (10 ng/ml), and subsequently treated with actinomycin D for 0, 60 or 120 min (ActD; 10 mg/ml). Relative normalized expression is calculated relative to Rpl13a and HPRT1 as references and untreated, unstimulated control is set to 1. Right panel: Residual mRNA expression after actinomycin D treatment given in percent of respective untreated sample. Quantification is representative for independent experiments (n>3); ± s.d. unpaired t-test, *P < 0.01. **G)** qRT-PCR analysis of IL-8 mRNA from U2-OS cells stimulated for 60 min with IL-1β (10 ng/ml) or H_2O_2 (100 µM) and subsequently treated with actinomycin D for 0, 30, 60 or 120 min (ActD; 10 mg/ml). Relative normalized expression is calculated as above. Quantification is representative for independent experiments (n>3); ± s.d. unpaired t-test, *P < 0.01. **H**) qRT-PCR of IL-8 mRNA from clonal U2-OS cells stably expressing a doxycycline-inducible shIKKβ left untreated or treated with doxycycline to induce IKKβ depletion. Cells were irradiated and after 60 min treated with ActD for 0, 30, 60 or for 120 min prior to harvest. Relative normalized expression is calculated with reference to two controls (Rpl13a and HPRT1). Residual mRNA expression after actinomycin D treatment given as percentage of respective untreated sample. Quantification is representative for independent experiments (n>3); ± s.d. unpaired t-test, *P < 0.01, ns not significant. **I)** Same as in H) with monoclonal U2-OS cells stably expressing a doxycycline-inducible shEDC4 plasmid. **J)** Quantitative RT-PCR of IL-8 (top panel) mRNA from EDC4 knockdown cells transiently reconstituted with shRNA-resistant wildtype EDC4 or phosphorylation-deficient EDC4 mutant (SA); ± s.d. unpaired t-test, *P < 0.01, ns not significant.

Figure S7. IKK/EDC4 regulates mRNA stability of both NF-κB-dependent and -independent transcripts. A) qRT-PCR of JunB (top, left panel), BAMBI (top, middle panel), IκBα (top, right panel) and IL-8 (bottom, left panel) mRNA from clonal U2-OS cells stably expressing a doxycycline-inducible shIKKβ left untreated (wt ut – blue, wt IR - green) or treated with doxycycline to induce IKKβ depletion ($IKK\beta^{sh}$ ut – red, $IKK\beta^{sh}$ IR - orange). Cells were irradiated and after 60 min treated with ActD for 0, 30, 60 or for 120 min prior to harvest. Relative normalized expression is calculated with reference to two controls (Rpl13a and HPRT1) and mRNA expression levels at 0 min ActD are set to 100 %. Bottom, right panel: comparison of fold change in residual mRNA expression 120 min after ActD treatment. Quantification is representative for independent experiments (n>3); ± s.d. unpaired t-test, *P < 0.01. **B)** Same as in **A)** with monoclonal U2-OS cells stably expressing a doxycycline-inducible shEDC4 plasmid. **C)** Quantitative RT-PCR of IL-8, BAMBI, DUSP1, JunB and NFKBIA mRNA from CRISPR control (CRISPRv2) or EDC4 knockout cells transiently reconstituted with wildtype EDC4, phosphorylation-deficient EDC4 mutant (SA) or empty vector as control (ctrl). Cells were left

unstimulated (solid line) or irradiated (stippled line) and after 60 min treated with ActD for 0, 60 or for 120 min prior to harvest. Statistical significance determined by unpaired t-test, *P < 0.05, ns not significant. **D)** Quantitative RT-PCR of IL-8, BAMBI, NFKBIA, JunB and DUSP1 mRNA of clonal U2-OS cells stably expressing a doxycycline-inducible shIKKβ left untreated (wt ut – blue, wt TNFα - green) or treated with doxycycline to induce IKKβ depletion ($IKK\beta^{sh}$ ut – red, $IKK\beta^{sh}$ TNFα - orange). Cells were left unstimulated or treated with TNFα (10 ng/ml) and after 30 min treated with ActD for 0, 60 or for 120 min prior to harvest. Relative normalized expression is calculated with reference to two controls (Rpl13a and HPRT1). Displayed are fold changes in residual mRNA expression 60 and 120 min after ActD treatment. Quantification is representative for independent experiments (n=2); ± s.d. unpaired t-test, *P < 0.05. **E)** U2-OS cells were transfected with siRNA against EDC4 (EDC4_si), IKKß (IKK_si), both (EDC4/IKK_si) or control siRNA (scr_si). Cells were left unstimulated (ut) or irradiated (20 Gy) and after 60 min treated with ActD for 0, 60 or for 120 min prior to harvest. Relative normalized expression is calculated with reference to two controls (Rpl13a and HPRT1). Displayed are fold changes in residual mRNA expression 60 and 120 min after ActD treatment. Quantification is representative for independent experiments (n=2); ± s.d. unpaired t-test, *P < 0.05.

Figure S8. Regulation of ARE-containing transcripts via IKK-EDC4 axis and contribution of p38, JNK and MEK1/2 kinases to mRNA regulation following DNA damage. A) RT-PCR of firefly reporter mRNA from wt, EDC4- or IKK-KO cells transiently expressing pEZX-MT01 ARE reporter constructs bearing 0, 5 or 7 repeats of the ARE motif. Relative normalized expression is calculated with reference to intrinsic control (Renilla) ± s.d. unpaired t-test, *P < 0.05, ns not significant. **B)** U2-OS cells were pre-treated with DMSO or p38 (SB203580), JNK (SP600125) or MEK1/2 (PD0325901) inhibitor and whole cell extracts (WCE) were prepared from unstimulated and irradiated cells (20 Gy, 60 min). WCE were analysed by SDS-PAGE using antibodies against phosphorylated and total protein of the kinases and their substrates (cJun – JNK substrate, Hsp27 – p38 substrate, ERK1/2 – MEK1/2 substrate) to show the DNA damage-induced activation of the kinases and the efficiency of their inhibitors. **C)** qRT-PCR of IL-8 (top row), BAMBI (middle row) and TNFAIP3 (bottom row) mRNA from U2-OS cells pre-treated with p38, JNK or MEK1/2 inhibitor or DMSO. Pre-treated cells were irradiated and after 60 min treated with ActD for 0, 60 or for 120 min prior to harvest. Relative normalized expression is calculated with reference to two controls (Rpl13a and HPRT1) and mRNA expression levels at 0 min ActD are set to 100 %. Quantification is representative for independent experiments (n>3); ± s.d. unpaired t-test, *P < 0.01.

LIST OF SUPPLEMENTARY TABLES

Table EV1: **Induced Interaction partners of IKKγ in response to DNA damage**

Table EV2: **IKK phosphosites in recombinant EDC4 domains**

Table EV3: **IKK phosphosites in endogenous EDC4**

Table EV4: **IKKβ- and EDC4- regulated expression and stability of mRNAs in unstimulated and irradiated cells, gene lists and GO terms**

Table EV5: **Effect of irradiation on mRNA expression and stability, gene lists and GO terms**

Table EV6: **NF-κB target genes regulated post-transcriptionally by IKK and/or EDC4**

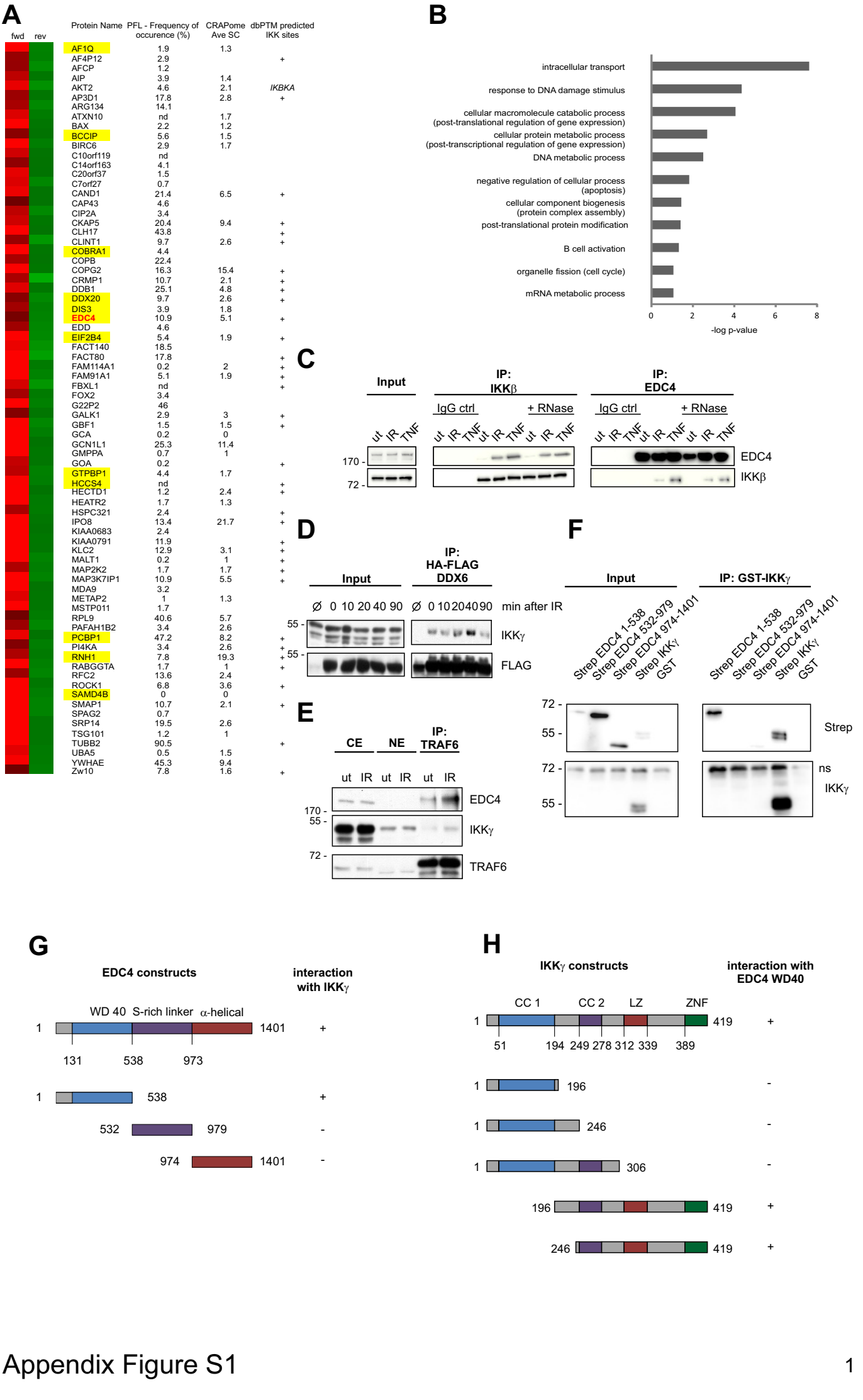
Table EV7: **Enrichment of ARE motifs in IKK- and EDC4-regulated targets**

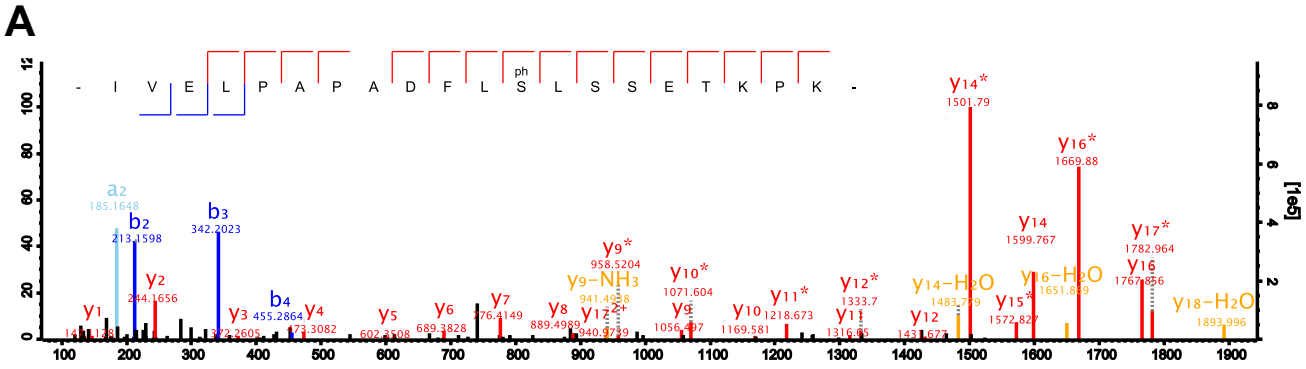
Table EV8: **ARE-containing transcripts regulated by IKK and EDC4**

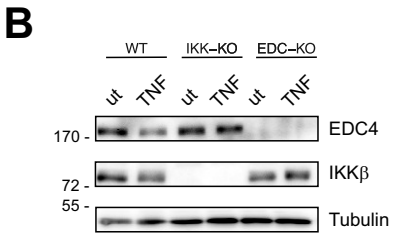
APPENDIX REFERENCES

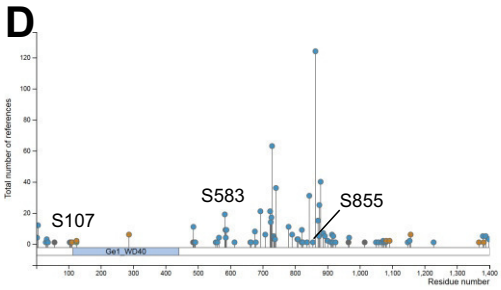
Stilmann M, Hinz M, Arslan SC, Zimmer A, Schreiber V, Scheidereit C (2009) A nuclear poly(ADP-ribose)-dependent signalosome confers DNA damage-induced IkappaB kinase activation. *Mol Cell* **36:** 365-378

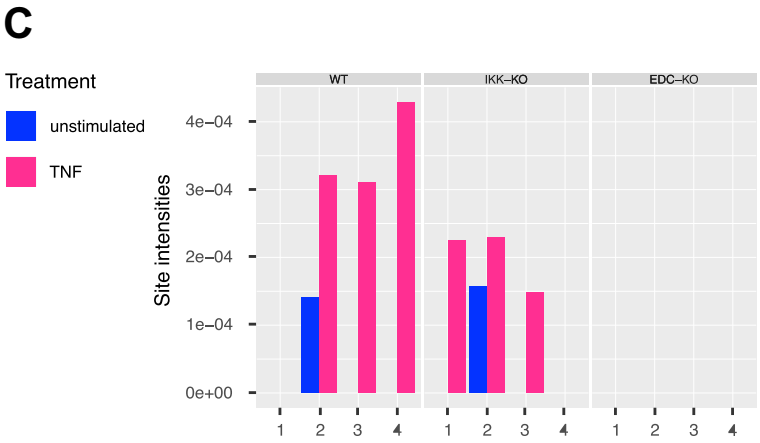
Supek F, Bosnjak M, Skunca N, Smuc T (2011) REVIGO summarizes and visualizes long lists of gene ontology terms. *PLoS One* **6:** e21800


A
fwd
rev
Protein Name
PFL - Frequency of occurence (%)
CRAPome Ave SC
dbPTM predicted IKK sites
AF1Q 1.9 1.3
AF4P12 2.9 +
AFCP 1.2
AIP 3.9 1.4
AKT2 4.6 2.1 IKBKA
AP3D1 17.8 2.8 +
ARG134 14.1
ATXN10 nd 1.7
BAX 2.2 1.2
BCCIP 5.6 1.5
BIRC6 2.9 1.7
C10orf119 nd
C14orf163 4.1
C20orf37 1.5
C7orf27 0.7
CAND1 21.4 6.5 +
CAP43 4.6
CIP2A 3.4
CKAP5 20.4 9.4 +
CLH17 43.8 +
CLINT1 9.7 2.6 +
COBRA1 4.4
COPB 22.4
COPG2 16.3 15.4 +
CRMP1 10.7 2.1 +
DDB1 25.1 4.8 +
DDX20 9.7 2.6 +
DIS3 3.9 1.8
EDC4 10.9 5.1 +
EDD 4.6
EIF2B4 5.4 1.9 +
FACT140 18.5
FACT80 17.8 +
FAM114A1 0.2 2 +
FAM91A1 5.1 1.9 +
FBXL1 nd +
FOX2 3.4
G22P2 46
GALK1 2.9 3 +
GBF1 1.5 1.5 +
GCA 0.2 0
GCN1L1 25.3 11.4
GMPPA 0.7 1
GOA 0.2 +
GTPBP1 4.4 1.7
HCCS4 nd +
HECTD1 1.2 2.4 +
HEATR2 1.7 1.3
HSPC321 2.4 +
IPO8 13.4 21.7 +
KIAA0683 2.4
KIAA0791 11.9 +
KLC2 12.9 3.1 +
MALT1 0.2 1 +
MAP2K2 1.7 1.7 +
MAP3K7IP1 10.9 5.5 +
MDA9 3.2
METAP2 1 1.3
MSTP011 1.7
RPL9 40.6 5.7
PAFAH1B2 3.4 2.6
PCBP1 47.2 8.2 +
PI4KA 3.4 2.6 +
RNH1 7.8 19.3 +
RABGGTA 1.7 1 +
RFC2 13.6 2.4
ROCK1 6.8 3.6 +
SAMD4B 0 0
SMAP1 10.7 2.1 +
SPAG2 0.7
SRP14 19.5 2.6
TSG101 1.2 1
TUBB2 90.5 +
UBA5 0.5 1.5
YWHAE 45.3 9.4
Zw10 7.8 1.6 +
B
intracellular transport
response to DNA damage stimulus
cellular macromolecule catabolic process (post-translational regulation of gene expression)
cellular protein metabolic process (post-transcriptional regulation of gene expression)
DNA metabolic process
negative regulation of cellular process (apoptosis)
cellular component biogenesis (protein complex assembly)
post-translational protein modification
B cell activation
organelle fission (cell cycle)
mRNA metabolic process
0 2 4 6 8
-log p-value
C
Input
IP: IKKβ
IP: EDC4
IgG ctrl
+ RNase
ut IR TNF
170 -
72 -
EDC4
IKKβ
D
Input
IP: HA-FLAG DDX6
Ø 0 10 20 40 90 Ø 0 10 20 40 90 min after IR
55 -
IKKγ
FLAG
E
CE
NE
IP: TRAF6
ut IR ut IR ut IR
170 -
55 -
72 -
EDC4
IKKγ
TRAF6
F
Input
IP: GST-IKKγ
Strep EDC4 1-538
Strep EDC4 532-979
Strep EDC4 974-1401
Strep IKKγ
GST
72 -
55 -
Strep
ns
IKKγ
G
EDC4 constructs
interaction with IKKγ
WD 40 S-rich linker α-helical
1 1401 +
131 538 973
1 538 +
532 979 -
974 1401 -
H
IKKγ constructs
interaction with EDC4 WD40
CC 1 CC 2 LZ ZNF
1 419 +
51 194 249 278 312 339 389
1 196 -
1 246 -
1 306 -
196 419 +
246 419 +

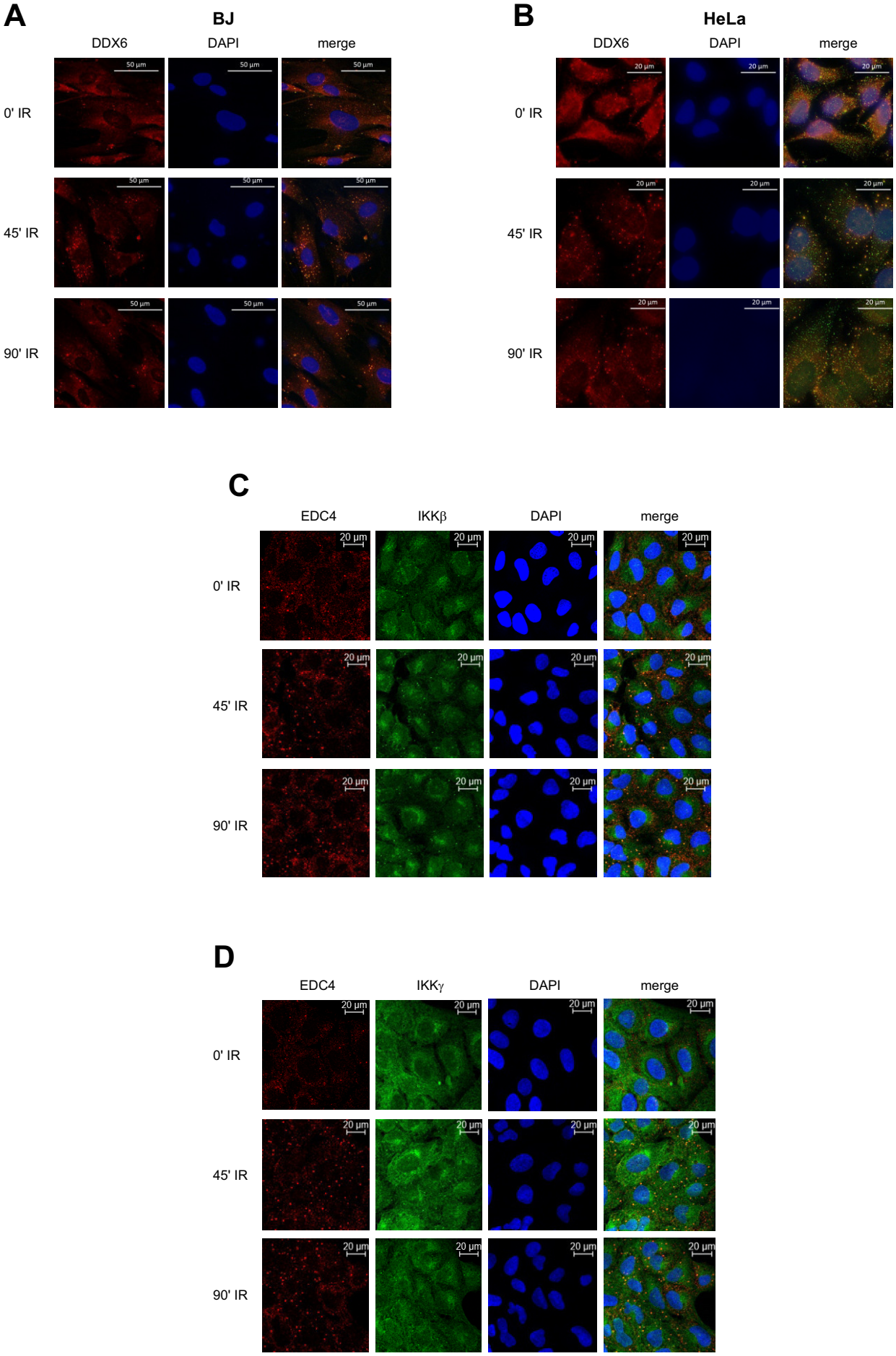


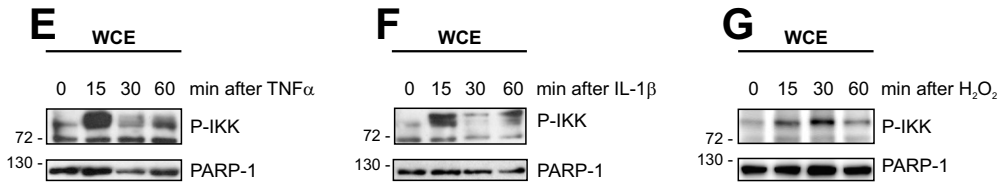


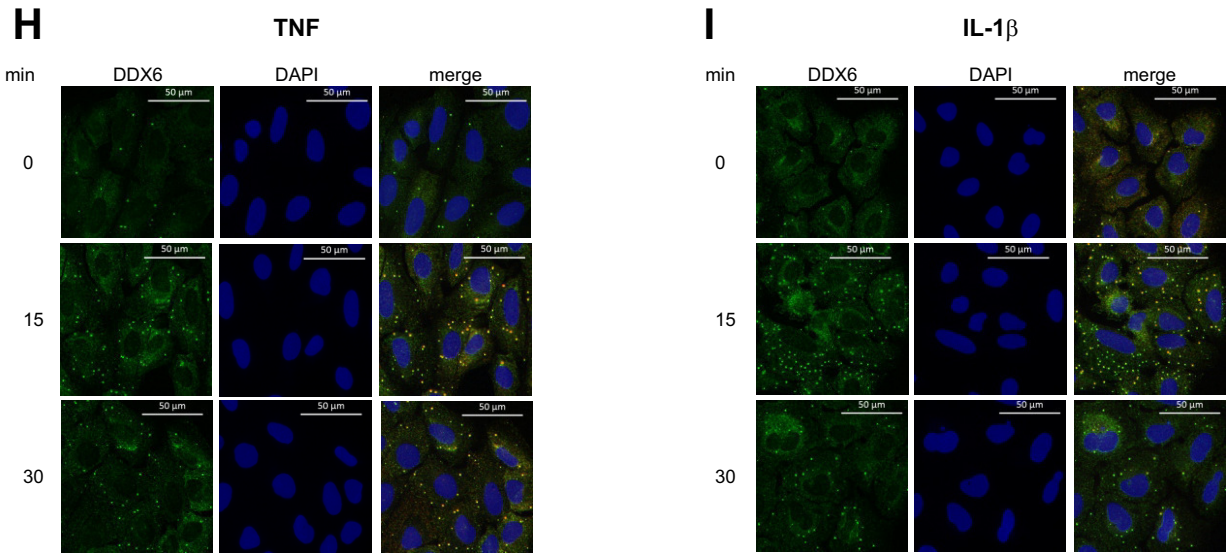


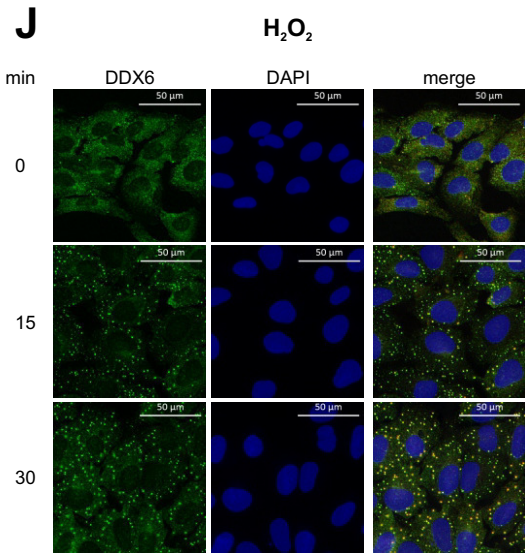


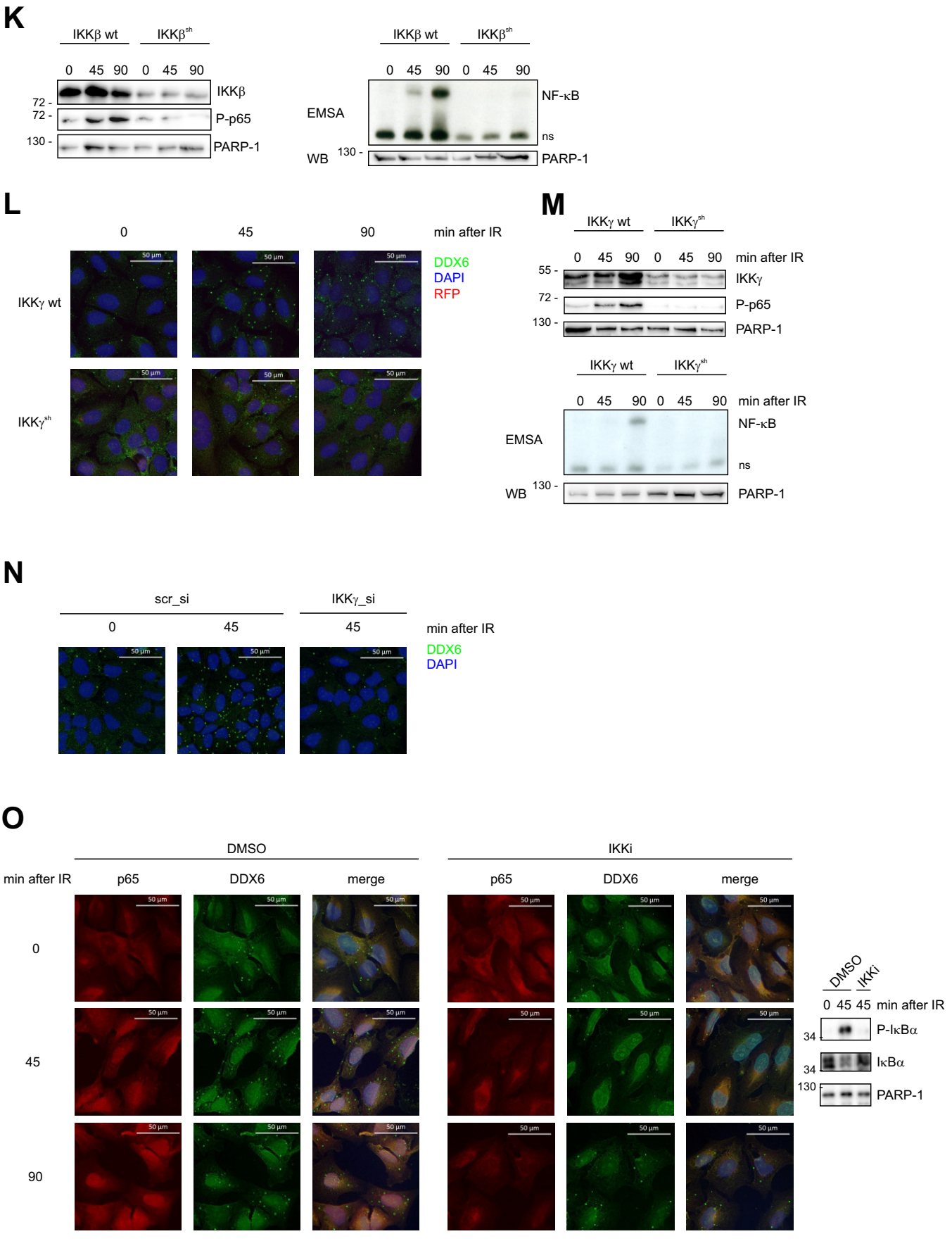
Appendix Figure S2

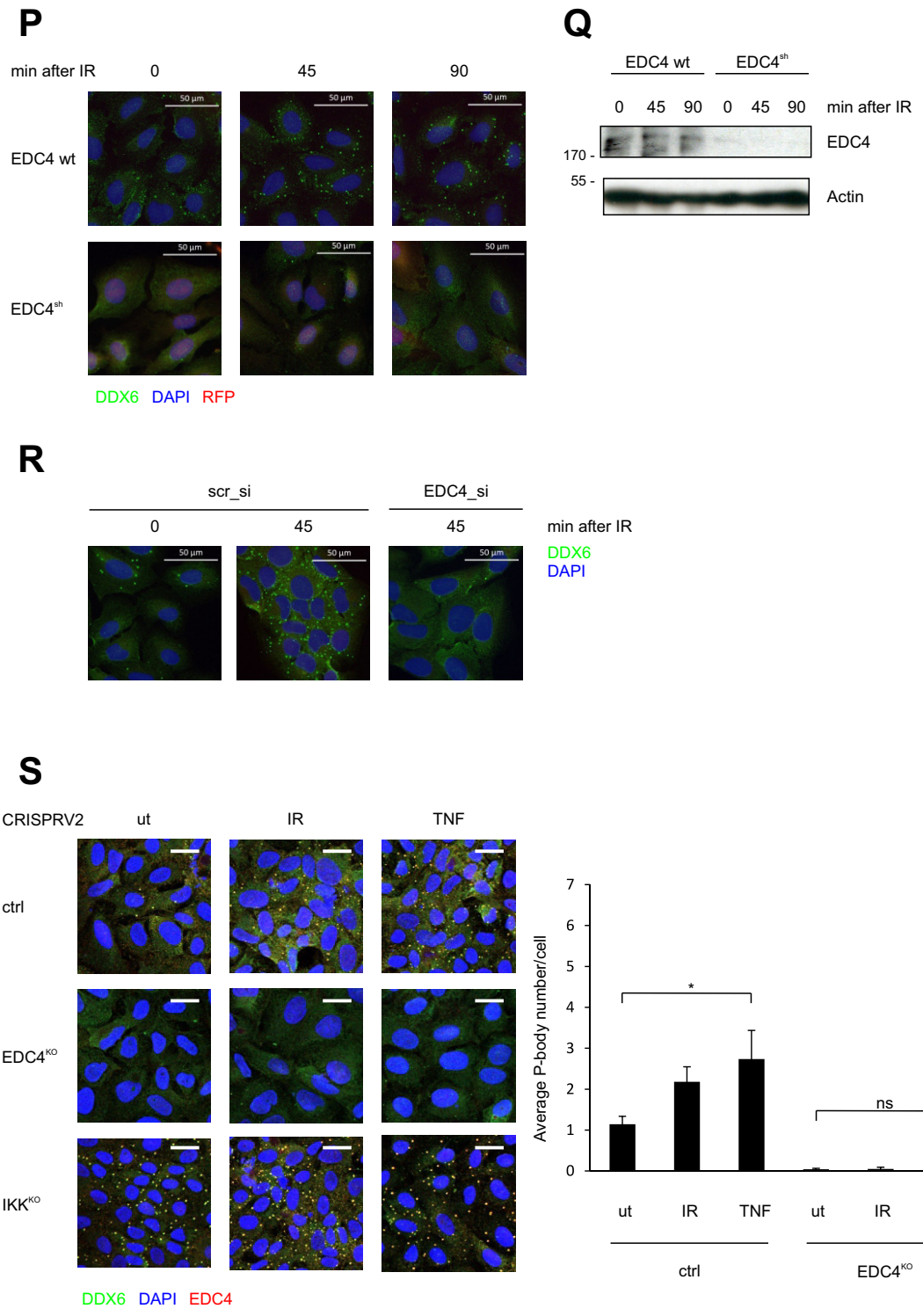

A
BJ
DDX6
DAPI
merge
0' IR
45' IR
90' IR
50 µm
B
HeLa
DDX6
DAPI
merge
0' IR
45' IR
90' IR
20 µm
C
EDC4
IKKβ
DAPI
merge
0' IR
45' IR
90' IR
20 µm
D
EDC4
IKKγ
DAPI
merge
0' IR
45' IR
90' IR
20 µm

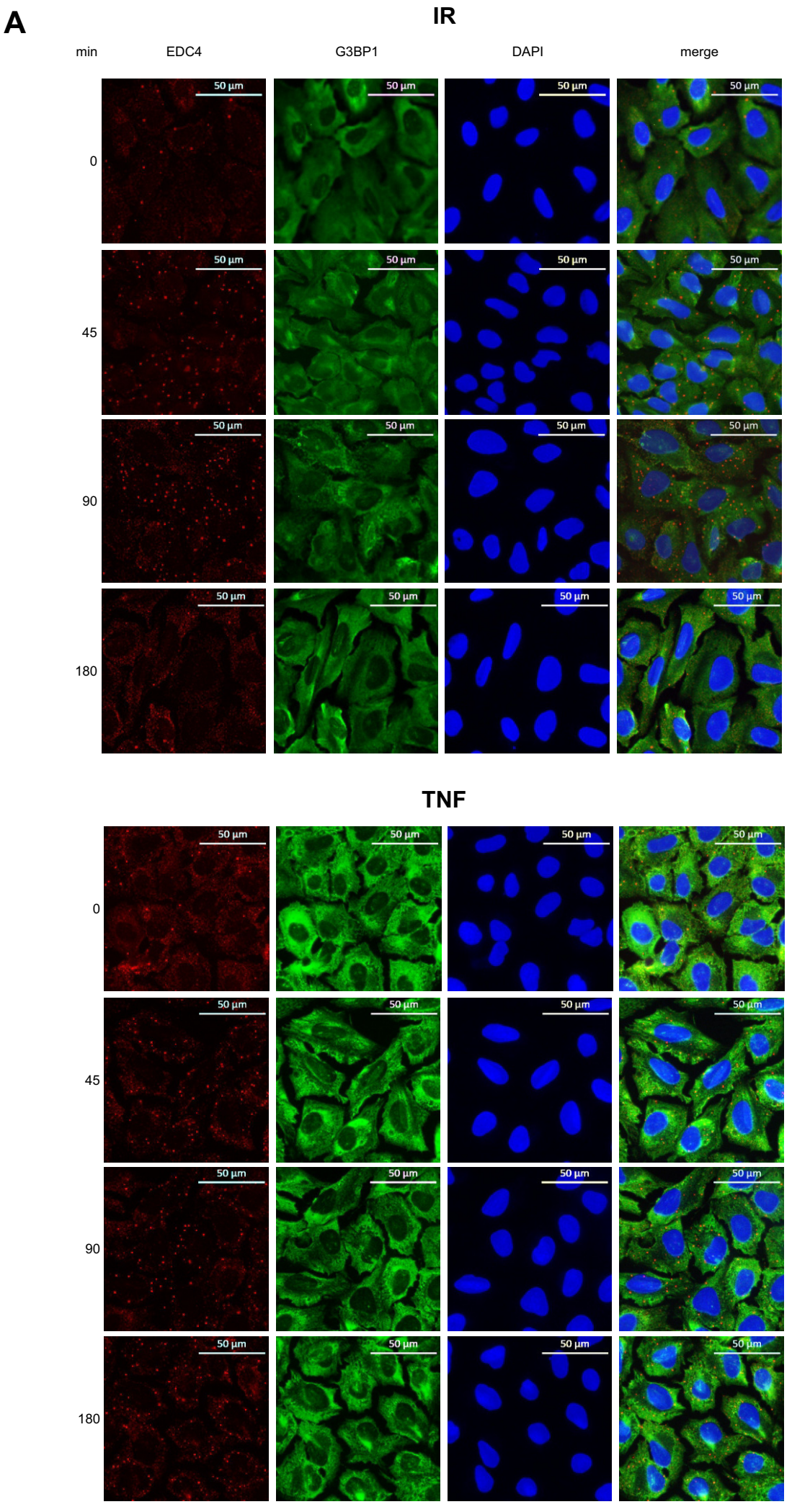

E
WCE
0 15 30 60 min after TNFα
72 -
130 -
P-IKK
PARP-1
F
WCE
0 15 30 60 min after IL-1β
72 -
130 -
P-IKK
PARP-1
G
WCE
0 15 30 60 min after H2O2
72 -
130 -
P-IKK
PARP-1


H
TNF
min
DDX6
DAPI
merge
0
15
30
I
IL-1β
min
DDX6
DAPI
merge
0
15
30


J
H2O2
min
DDX6
DAPI
merge
0
15
30

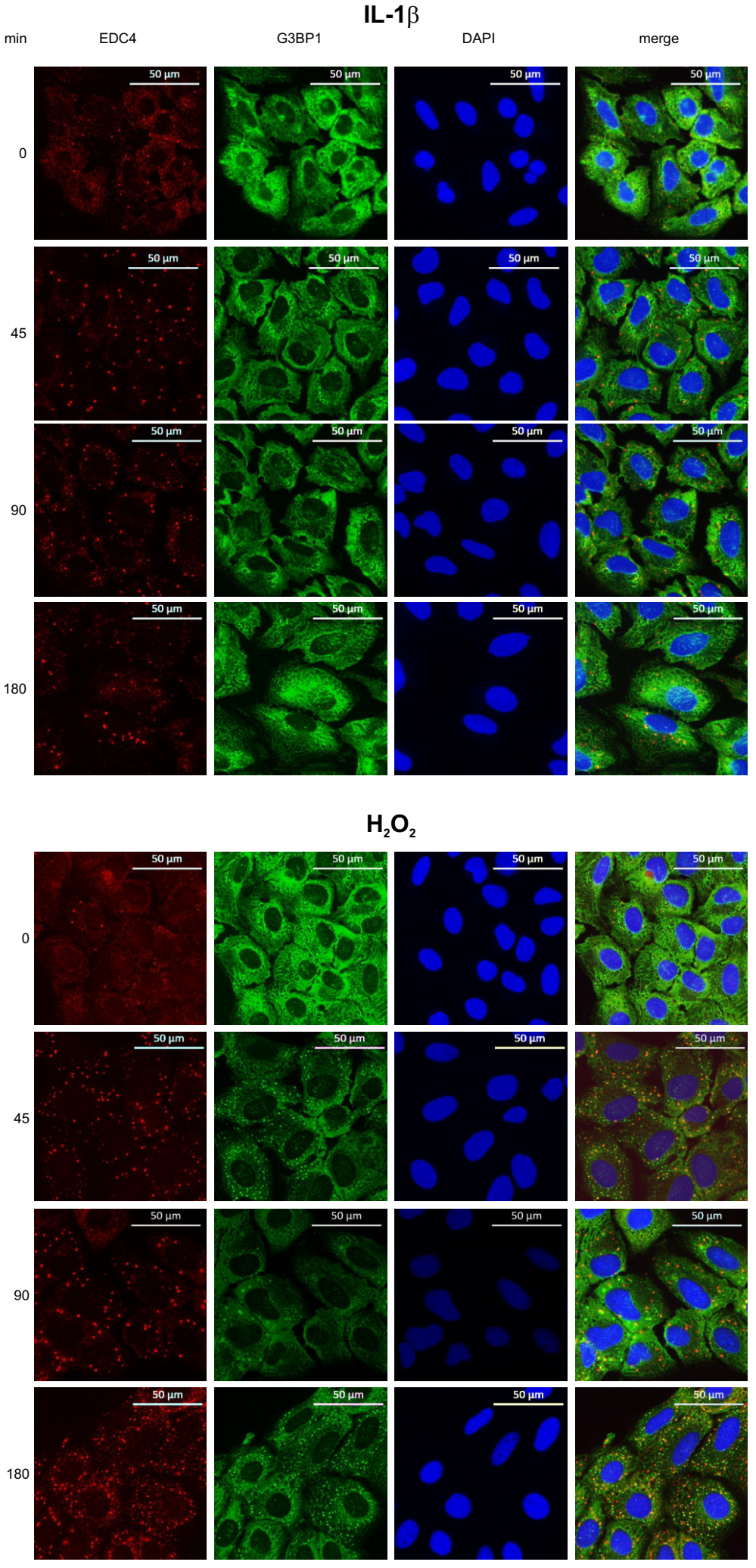

K
IKKβ wt
IKKβsh
0 45 90 0 45 90
IKKβ
P-p65
PARP-1
72 -
72 -
130 -
EMSA
WB
NF-κB
ns
L
min after IR
IKKγ wt
IKKγsh
DDX6
DAPI
RFP
50 µm
M
IKKγ wt
IKKγsh
IKKγ
55 -
N
scr_si
IKKγ_si
O
DMSO
IKKi
p65
DDX6
merge
P-IκBα
IκBα
34 -
130 -


P
min after IR
0
45
90
EDC4 wt
EDC4sh
50 µm
DDX6 DAPI RFP
Q
EDC4 wt
EDC4sh
0 45 90 0 45 90 min after IR
170 -
55 -
EDC4
Actin
R
scr_si
EDC4_si
0
45
45
min after IR
DDX6
DAPI
S
CRISPRV2
ut
IR
TNF
ctrl
EDC4KO
IKKKO
DDX6 DAPI EDC4
Average P-body number/cell
0
1
2
3
4
5
6
7
*
ns
ns
ut IR TNF ut IR TNF ut IR TNF
ctrl
EDC4KO
IKKKO



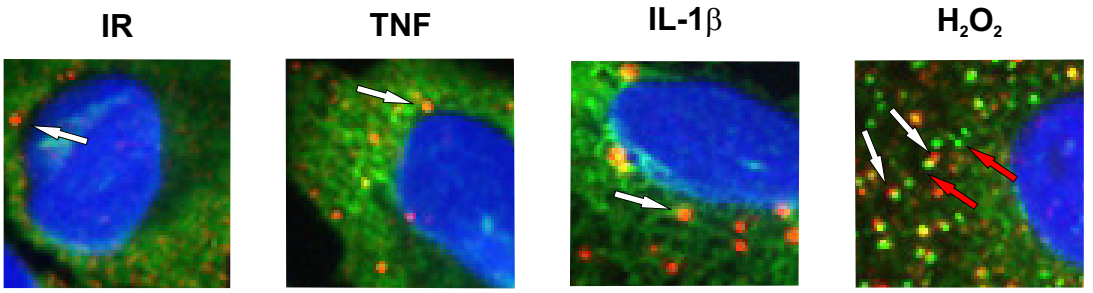
Appendix Figure S4

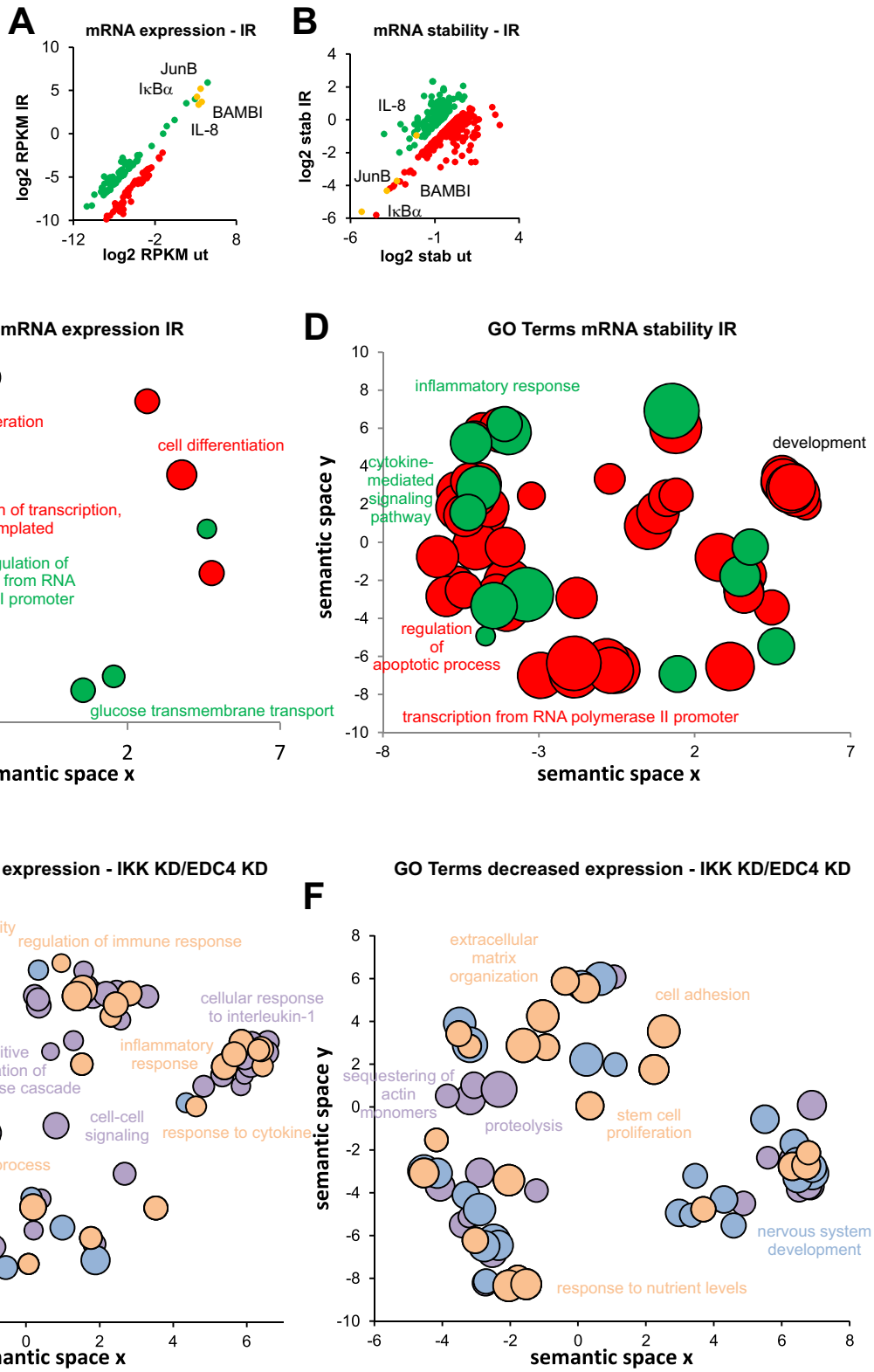
Appendix Figure S4A continued



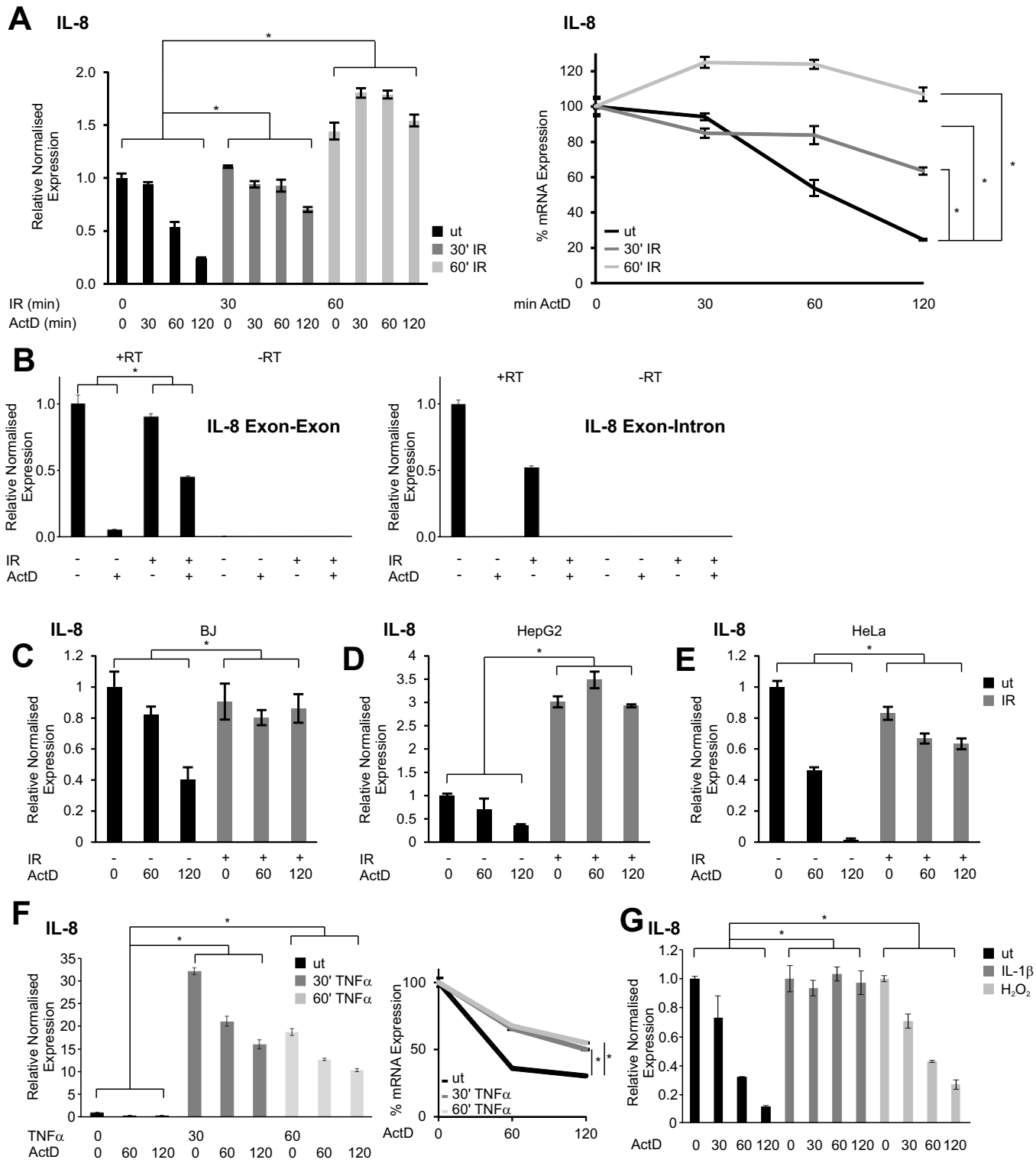
Appendix Figure S4

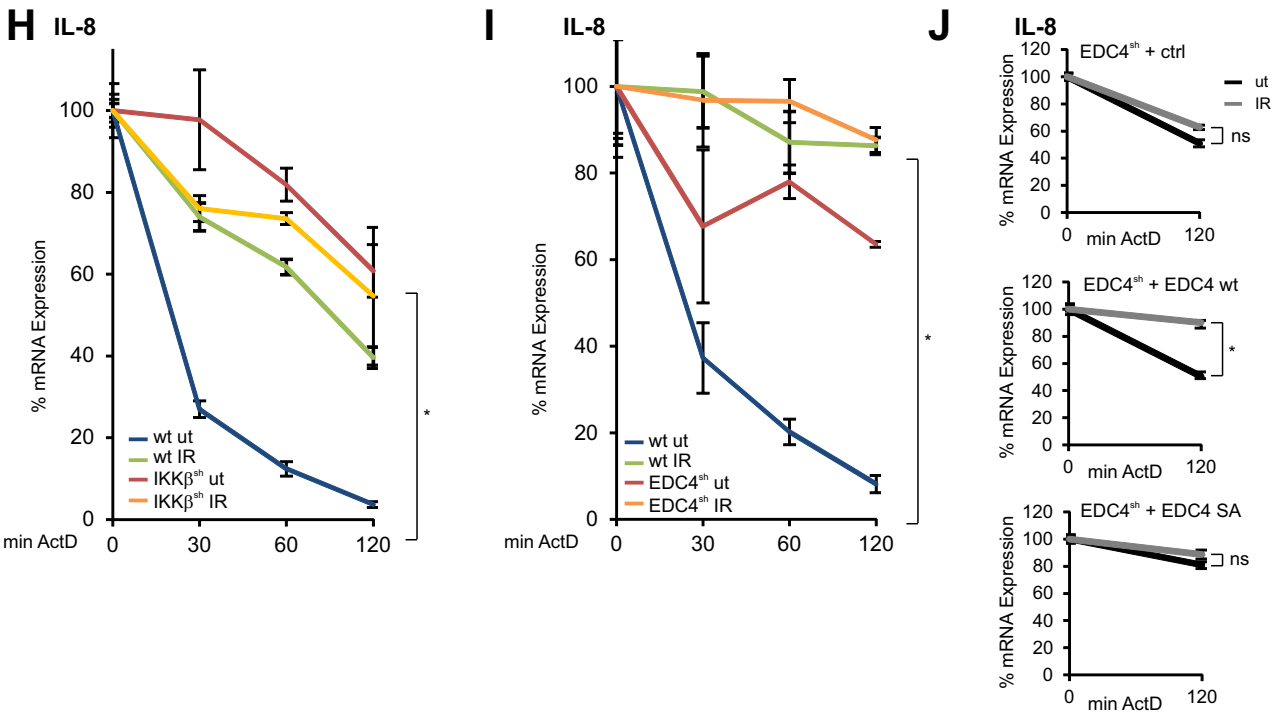
B





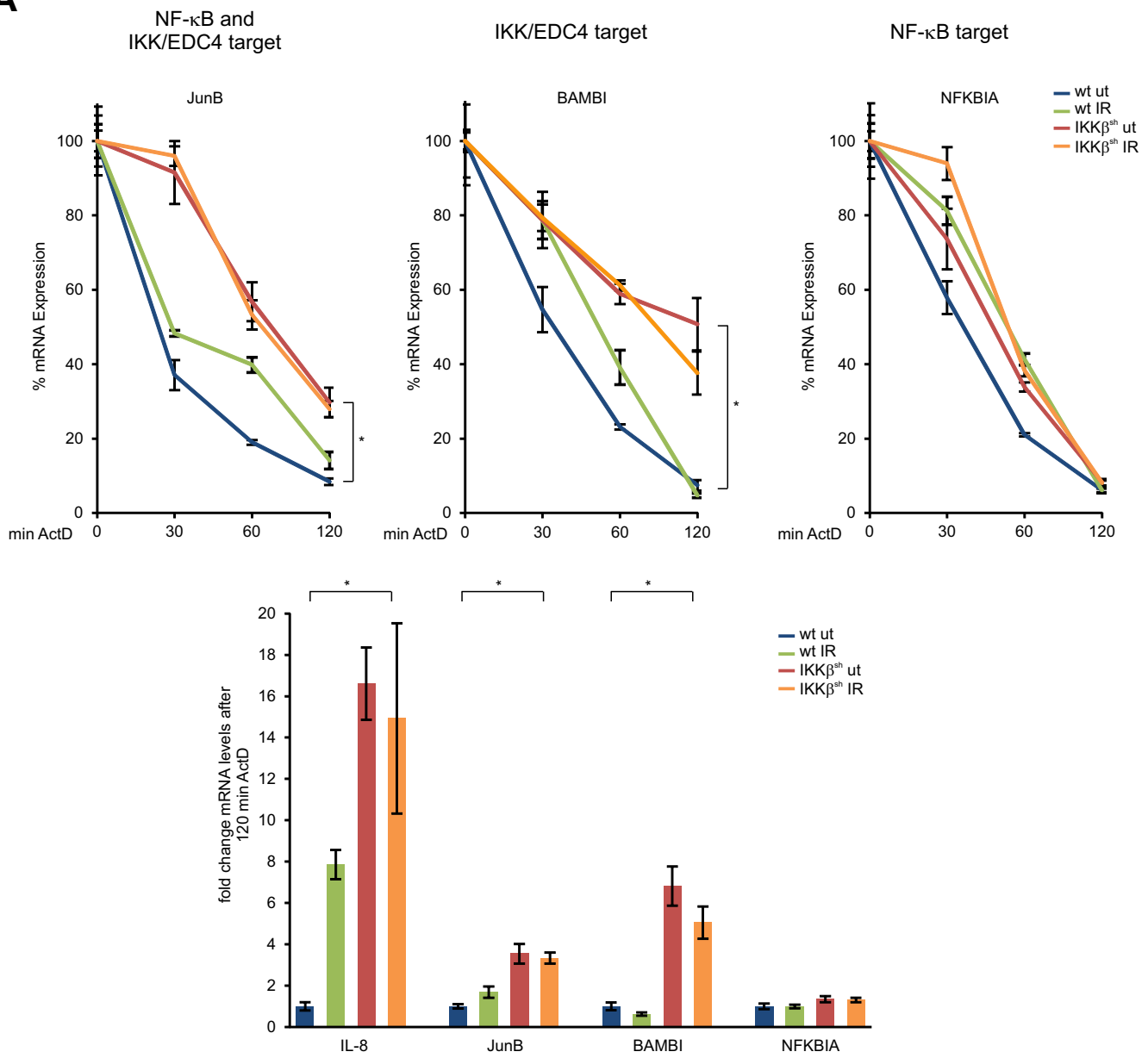
Appendix Figure S5


A
IL-8
Relative Normalised Expression
ut
30' IR
60' IR
IR (min)
ActD (min)
% mRNA Expression
min ActD
B
+RT
-RT
IL-8 Exon-Exon
IL-8 Exon-Intron
IR
ActD
C
BJ
D
HepG2
E
HeLa
F
TNFα
30' TNFα
60' TNFα
G
IL-1β
H2O2

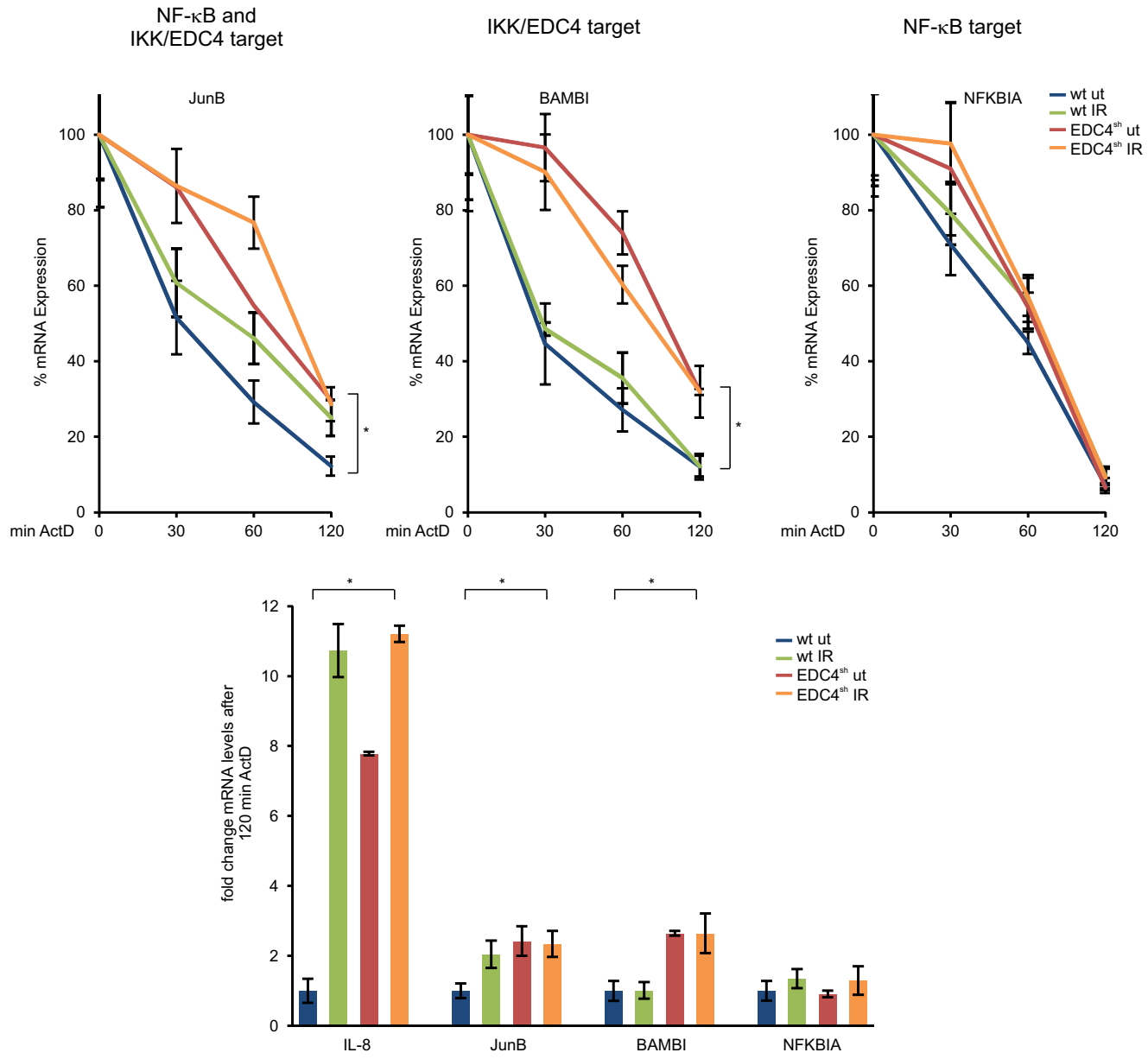


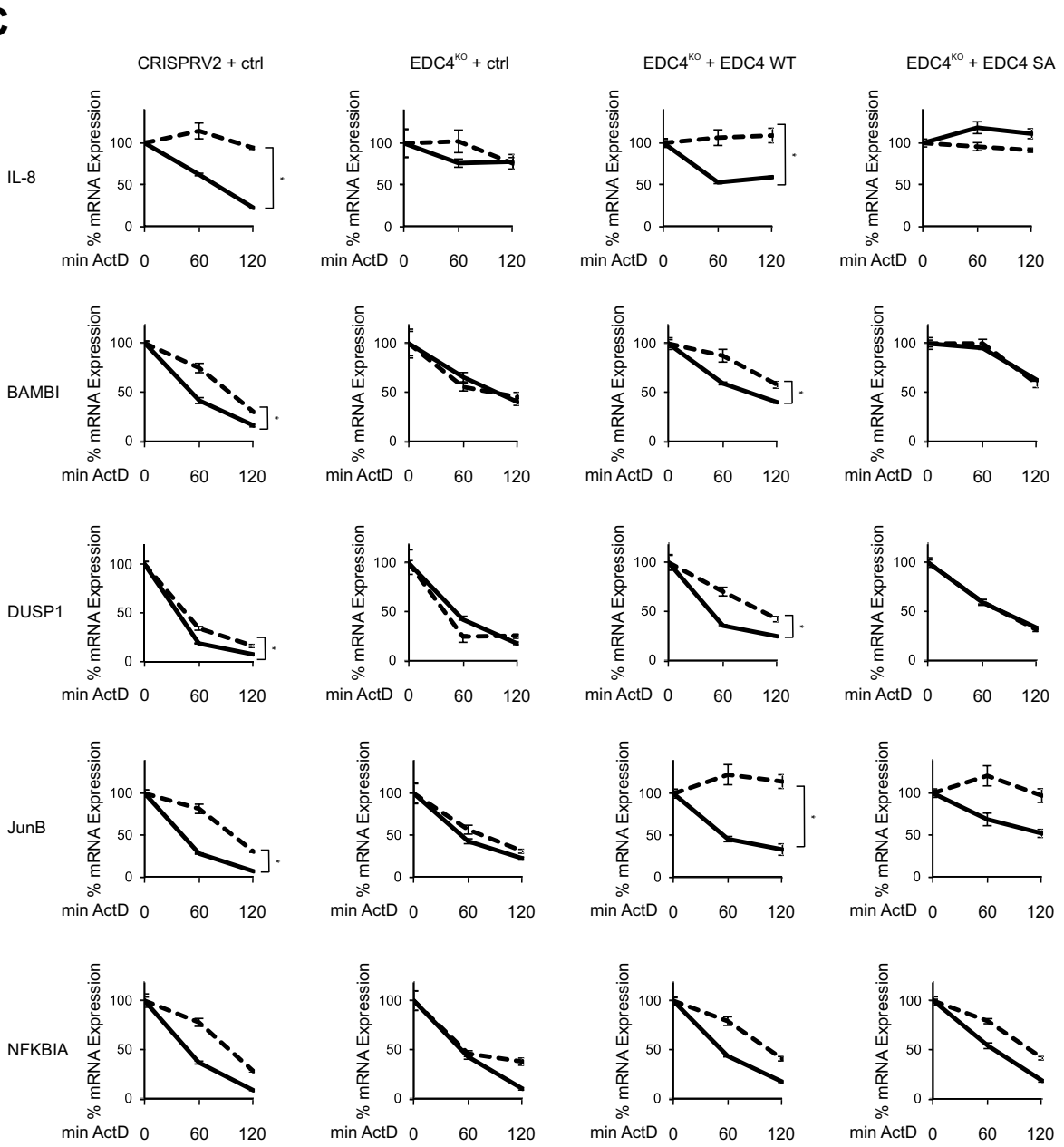
Appendix Figure S6

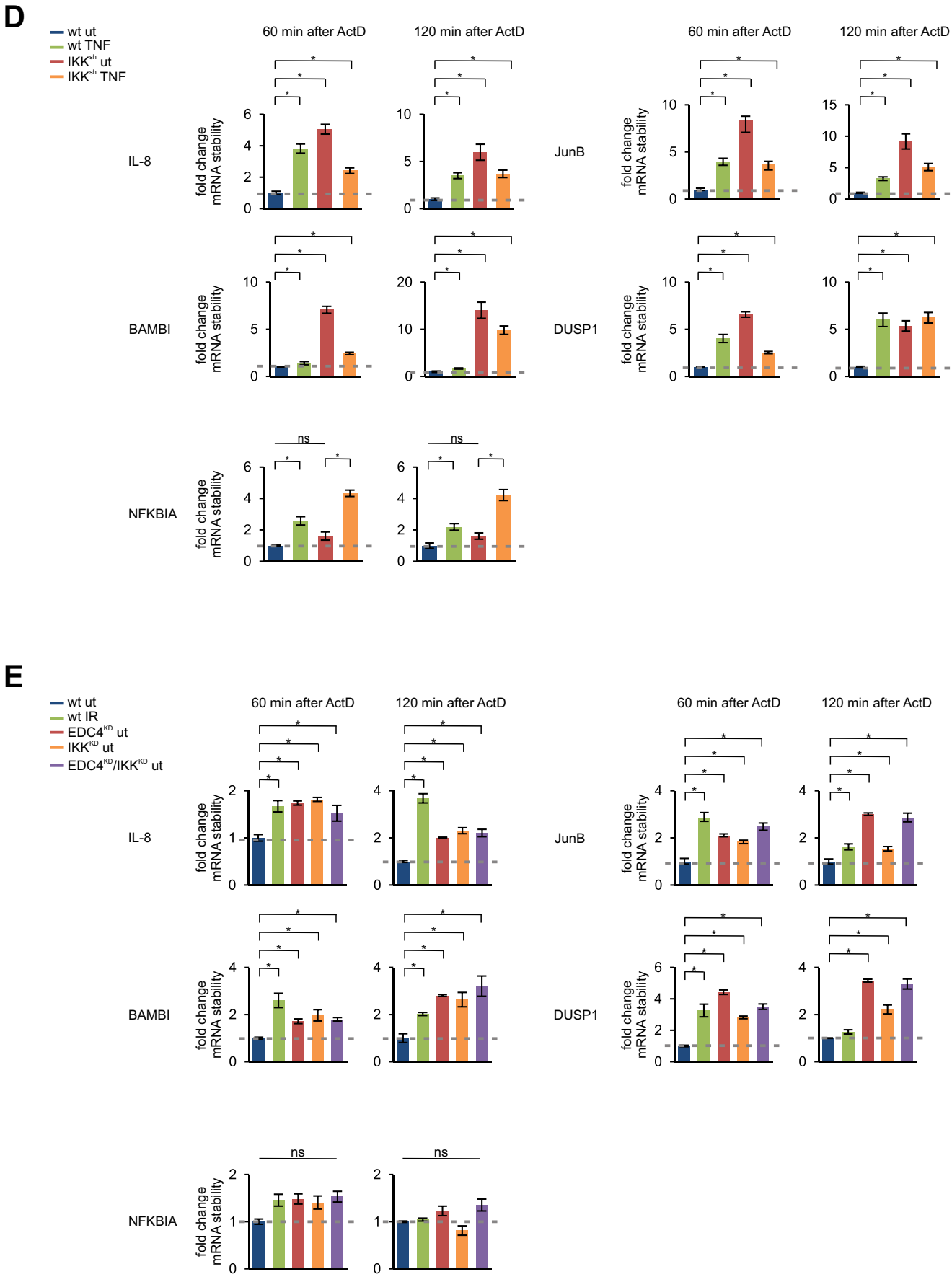
A


NF-κB and
IKK/EDC4 target
IKK/EDC4 target
NF-κB target
JunB
BAMBI
NFKBIA
wt ut
wt IR
IKKβsh ut
IKKβsh IR
% mRNA Expression
min ActD
0
30
60
120
fold change mRNA levels after
120 min ActD
IL-8
JunB
BAMBI
NFKBIA

B

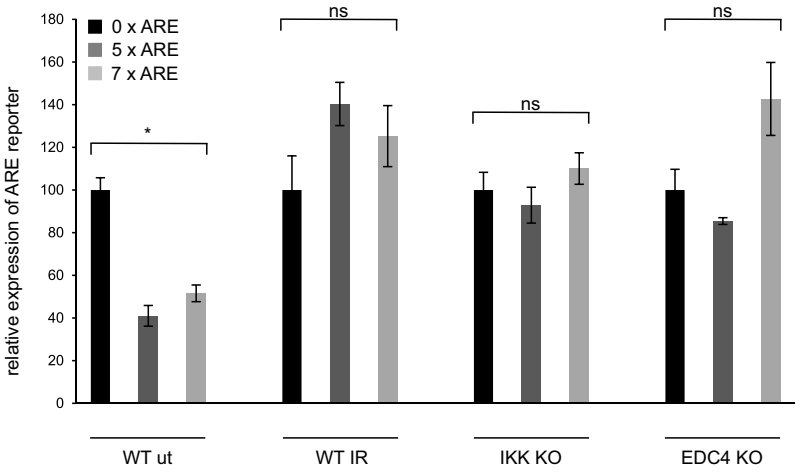

NF-κB and IKK/EDC4 target
IKK/EDC4 target
NF-κB target
JunB
BAMBI
NFKBIA
wt ut
wt IR
EDC4sh ut
EDC4sh IR
% mRNA Expression
min ActD
0
30
60
120
*
fold change mRNA levels after 120 min ActD
IL-8
JunB
BAMBI
NFKBIA


C
CRISPRV2 + ctrl
EDC4KO + ctrl
EDC4KO + EDC4 WT
EDC4KO + EDC4 SA
IL-8
BAMBI
DUSP1
JunB
NFKBIA
% mRNA Expression
100
50
0
min ActD 0 60 120
*

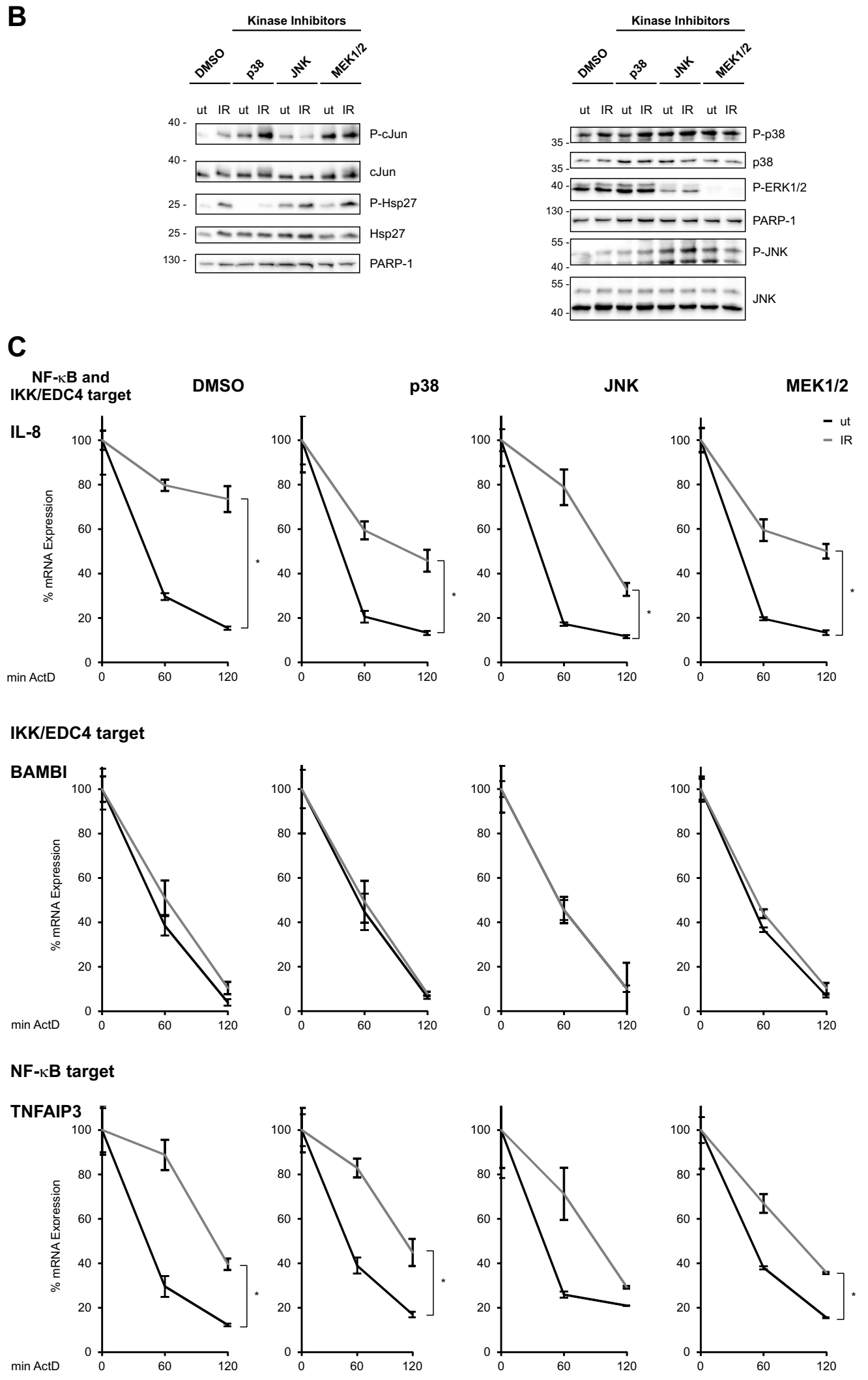


Appendix Figure S7

A



Appendix Figure S8



Appendix Figure S8