SUPPLEMENTARY FIGURE LEGENDS

Figure S1

(A) Cell proliferation. Presenescent HCA2 cells (PRE) were treated with 10 Gy X-ray (XRA) and counted at the indicated intervals thereafter.

(B) SA-βgal staining. PRE cells were irradiated, allowed to senesce (SEN(XRA)) for 8-10 d, fixed, and stained for SA-βgal.

(C) XRA does not induce a rapid, transient increase in p38MAPK phosphorylation. PRE cells were either irradiated (XRA) or treated with 100 ng/mL LPS. Whole cell lysates were collected at the indicated intervals thereafter and analyzed by western blotting.

(D) p38MAPK inhibition decreases secreted IL-6 in SEN(XRA) WI-38 cells. Presenescent WI-38 cells (PRE) were irradiated and allowed to senesce (SEN(XRA)). Conditioned media (CM) were collected and analyzed by ELISA. SB: p38MAPK was inhibited by SB023580 for 48 h prior to CM collection.

(E) p38MAPK inhibition decreases secreted IL-6 by SEN(REP) HCA2 and WI-38 cells. PRE cells were cultured to replicative senescence (SEN(REP)), and CM were collected and analyzed by ELISA. SB: p38MAPK was inhibited by SB023580 for 48 h prior to CM collection.

(F) Efficacy of p38MAPKα shRNAs. PRE cells were infected with a lentivirus expressing either of two shRNAs against p38α (shp38α) or an shRNA against GFP (shGFP; control), and selected. Cells were irradiated and allowed to senesce (SEN(XRA)). Whole cell lysates were collected and analyzed by western blotting for the indicated p38MAPK isoforms and tubulin (control).

(G) p38MAPK inhibition suppresses the SEN(XRA) SASP. Shown are factors for which the change between SEN(XRA)+SB and SEN(XRA) was statistically significant ($p<0.05$) (marked by asterisks in Figure 1E). Values indicate the $\log_2$-fold decrease from SEN(XRA) levels.

(H) p38MAPK inhibition slightly increases secreted levels of some proteins. Factors for which p38MAPK inhibition by SB023580 (SB) increased secreted levels, relative to untreated SEN(XRA) cells. Values indicate the $\log_2$-fold change between SEN(XRA)+SB and SEN(XRA). None of the factors increased by SB were components of the SEN(XRA) SASP.

(I) p38MAPK inhibition decreases MMP3 but not MMP1 secreted by SEN(XRA) cells. PRE cells were irradiated and allowed to senesce for 8 d (SEN(XRA)). CM were collected

and analyzed by ELISA. SB: p38MAPK was inhibited by SB023580 for 48 h prior to CM collection (2d), or p38MAPK was inhibited by SB203580 starting 48 h prior to irradiation and continuing until CM collection (cont).

Figure S2

(A) p38MAPK inhibition suppresses the SEN(RAS) SASP. PRE cells were infected as described in Figure 2A. +SB: p38MAPK was inhibited by SB203580 for 48 h prior to CM collection. Proteins secreted by the indicated cells were detected by antibody arrays. Shown are the 83 factors for which the SEN(RAS) level was significantly increased over PRE. For each protein, PRE and SEN(RAS) values were averaged to generate the baseline. Signals above baseline are yellow; signals below baseline are blue. The heat map key show $\log_2$-fold changes from baseline. The relationship between samples is indicated graphically by hierarchical clustering (top). Asterisks indicate the factors significantly decreased by p38MAPK inhibition.

(B) p38MAPK inhibition decreases MMP1 and MMP3 secreted by SEN(RAS) cells. PRE cells were infected as described in Figure 2A. CM were collected and analyzed by ELISA. SB: p38MAPK was inhibited by SB023580 for 48 h prior to CM collection (2d), or p38MAPK was inhibited by SB203580 starting 48 h prior to irradiation and continuing until CM collection (cont).

(C) The SEN(XRA) and SEN(RAS) SASPs are similarly dependent on p38MAPK. The table lists SASP factors that are significantly increased over PRE control in both SEN(XRA) and SEN(RAS) cells. • indicates factors significantly decreased by p38MAPK inhibition in both SEN(XRA) and SEN(RAS) cells.

(D) Efficacy of p53 shRNAs. PRE cells were infected with a lentivirus expressing an shRNA against p53 (shp53) or GFP (shGFP; control) and selected. Whole cell lysates were collected and analyzed by western blotting for the indicated proteins.

(E) Amplified levels of IL-6, IL-8 and GM-CSF induced by p53 depletion are p38MAPK dependent. PRE cells were infected with a lentivirus expressing an shRNA against p53 (shp53) or GFP (shGFP; control), selected, and allowed to recover. Cells were then irradiated and CM was collected 3 d later and analyzed by ELISA. SB: p38MAPK was inhibited by SB203580 for 48 h prior to CM collection.

Figure S3

(A) MKK6EE induces SA-βgal expression. HCA2 and WI-38 cells were infected with a lentivirus expressing the constitutively active MAP kinase kinase 6 mutant (MKK6EE). Cells were selected, fixed 8 d after infection, and stained for SA-βgal. Presenescent controls (PRE) were infected with an insertless vector.

(B) MKK6EE suppresses BrdU incorporation. PRE cells were infected as described in (A), and 7 d later cultured with BrdU for 24 h, fixed, and immunostained for incorporated BrdU. BrdU-positive cells were quantified by CellProfiler. SB: p38MAPK was continuously inhibited with SB023580 beginning 48 h before infection.

(C) MKK6EE induces a p38MAPK-dependent senescence morphology. PRE cells were infected as described in (A) and photographed through a phase contrast microscope 8 d after infection. Representative images are shown. +SB: p38MAPK was continuously inhibited with SB023580 beginning 48 h before infection.

(D) MKK6EE increases secreted IL-6. PRE cells were infected as described in (A). CM were collected 8 d after infection and analyzed by ELISA. SB: p38MAPK was continuously inhibited with SB023580 beginning 48 h before infection.

(E) MKK6EE increases secreted MMP1 and MMP3 levels. PRE cells were infected as described in (A). CM were collected 8 d after infection and analyzed by ELISA.

Figure S4

(A) Effect of p38MAPK inhibition on 53BP1 foci formation and resolution. PRE cells were irradiated, fixed at the indicated intervals thereafter, and analyzed by immunostaining for 53BP1. Foci were quantitated using CellProfiler. Cells with ≥3 53BP1 foci/nucleus were scored. Error bars indicate the margin of error at 95% confidence. +SB: p38MAPK was continuously inhibited by SB203580 beginning 48 h before irradiation.

(B) Effect of p38MAPK inhibition on 53BP1 foci in replicatively senescent cells. PRE cells were cultured to replicative senescence (SEN(REP)), then fixed and analyzed by immunostaining for 53BP1. Foci were quantitated using CellProfiler. +SB: p38MAPK was inhibited by SB023580 for 6 d prior to fixation.

(C) Constitutive p38MAPK activation does not induce 53BP1 foci. Cells were fixed 8 d after MKK6EE expression (MKK6EE), 10 d after RAS expression (SEN(RAS)), 8 d after irradiation (SEN(XRA)), and after replicative senescence (69 population doublings) (SEN(REP)) and immunostained for 53BP1. Foci were quantified using CellProfiler. The data are identical to Figure 4B, displayed as a histogram.

(D) Oncogenic RAS, but not constituve p38MAPK activation, induces p53 phosphorylation. Cells were infected with a RAS lentivirus or an MKK6EE lentivirus. Whole cell lysates were collected at the indicated timepoints and analyzed by western blotting. p53-P (Ser15), p53 phosphorylated on residue Ser15.

(E) ATM or Chk2 depletion by RNAi. PRE cells were simultaneously infected with a lentivirus expressing MKK6EE and a lentivirus expressing an shRNA against ATM (shATM #12), CHK2 (shChk2 #2, shChk2 #12), or GFP (shGFP; control) and selected. Whole cell lysates were collected 8 d after infection and analyzed by western blotting for the indicated proteins. Presenescent controls (PRE) were infected with an insertless vector.

(F) SB203580 dose response – effect on p38MAPK signaling. PRE cells were irradiated (XRA) or infected with a lentivirus expressing MKK6EE (MKK6EE), and whole cell lysate was collected 8 d later and analyzed by western blotting. p38MAPK was continuously inhibited by SB203580 (SB) at the indicated concentrations beginning 48 h before irradiation/infection.

(G) SB203580 dose response – effect on IL-6 secretion. Cells were treated as in (E) and CM were collected 8 d later and analyzed by ELISA.

Figure S5

(A) p38MAPK inhibition decreases SASP mRNA levels in WI-38 cells. PRE WI-38 cells were irradiated and allowed to senesce (SEN(XRA)). Total RNA was extracted and mRNA levels for the indicated genes were analyzed by quantitative RT-PCR. For each gene, the four conditions were averaged to generate the baseline. Signals above baseline are red; signals below baseline are green. The heat map key shows $\log_2$-fold changes from baseline. +SB: p38MAPK was inhibited with SB203580 for 48 h prior to sample collection. p38MAPK inhibition significantly decreased ($p<0.05$) mRNA levels for all genes shown except GM-CSF.

(B) Decreased mRNA levels closely match decreased secreted protein levels. PRE cells were irradiated and allowed to senesce (SEN(XRA)), then CM and RNA were collected. mRNA levels for IL-6, IL-8 and GM-CSF were analyzed by quantitative RT-PCR; secreted protein levels were analyzed by ELISA. SEN(XRA) mRNA and protein levels were set to 1 for each factor. SB: p38MAPK was inhibited with SB203580 for 48 h prior to sample collection. For all factors, the fold decrease in mRNA level after p38MAPK inhibition was not significantly different from the fold decrease in protein level ($p>0.05$).

(C) MKK6EE increases mRNA levels of IL-6 and IL-8. PRE cells were infected with a lentivirus expressing MKK6EE and selected. Total RNA was extracted 8 d after infection and mRNA levels for the indicated genes were analyzed by quantitative RT-PCR. Presenescent controls (PRE) were infected with an insertless vector.

(D) NF-κB DNA binding activity increases in multiple types of senescence. PRE cells were induced to senesce (SEN) by the indicated stimuli, and whole cell lysates were collected and assayed for NF-κB DNA binding activity.

(E) NF-κB DNA binding increases in senescent WI-38 cells. PRE WI-38 cells were irradiated and allowed to senesce (SEN(XRA)). Whole cell lysates were collected and assayed for NF-κB DNA binding activity.

(F) NF-κB DNA binding activity kinetics after DNA damage. PRE cells were irradiated (XRA); whole cell lysates were collected at the indicated intervals thereafter and assayed for NF-κB DNA binding activity.

(G) Efficacy of RelA shRNAs. PRE cells were infected with a lentivirus expressing either of two shRNAs against RelA (shRelA #1, #2) or GFP (shGFP; control) and selected. Whole cell lysates were collected and analyzed by western blotting for the indicated NF-κB family members and tubulin (control).

(H) RelA depletion decreases secreted MMP1 and MMP3 levels. PRE cells were infected with lentiviruses expressing either of two shRNAs against RelA (shRNA #1, #2) or GFP (shGFP; control) and selected. Cells were then irradiated and allowed to senesce (SEN(XRA)). CM were collected and analyzed by ELISA.

(I) RelA depletion decreases secreted IL-6 levels in senescent HCA2 and WI-38 cells. PRE HCA2 (top) or WI-38 (bottom) cells were treated as described in (H). CM were collected and analyzed by ELISA.

(J) RelA depletion reduces the MKK6EE-induced SASP. PRE cells were infected with a lentivirus expressing either of two shRNAs against RelA (shRelA) or GFP (shGFP; control) and selected. Cells were then infected with a lentivirus lacking an insert (PRE) or expressing MKK6EE (MKK6EE). CM were collected 8d after infection and analyzed by ELISA.

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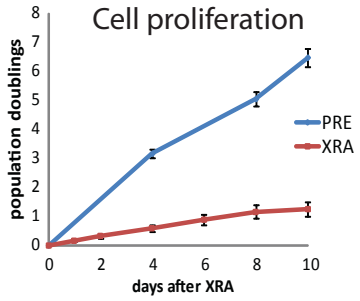
Figure S1

A



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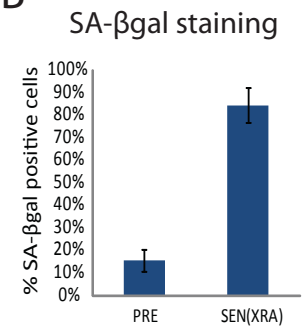

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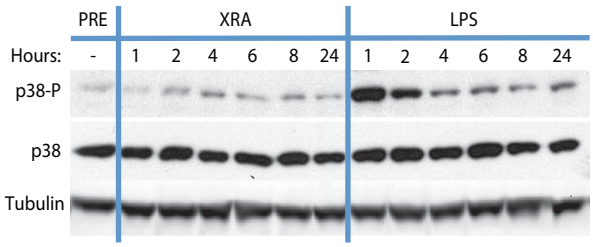

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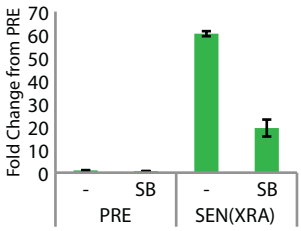

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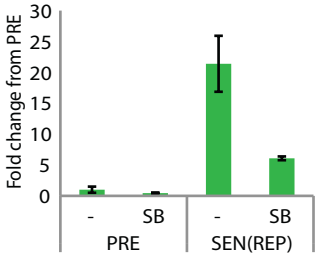

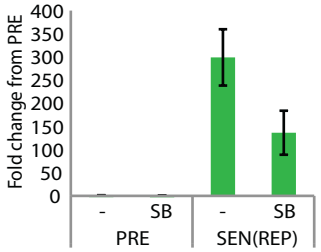

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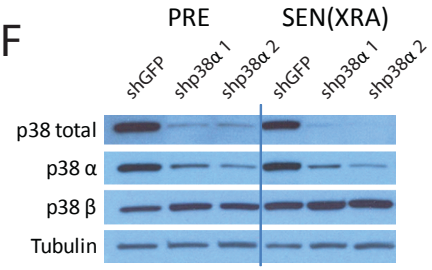

G

SASP proteins significantly reduced by p38 Inhibition

Factor	Log2 change from SEN(XRA)
GRO	-3.31
IL-6	-2.84
IL-8	-2.47
MCP-2	-2.43
MCP-3	-1.99
GCP-2	-1.35
GRO-alpha	-1.26
Angiogenin	-1.22
GM-CSF	-1.04
MCP-1	-0.99
CNTF	-0.91
GDNF	-0.90
IL-10	-0.87
IL-7	-0.80
MIG	-0.74
IL-2	-0.73
TGF-beta 1	-0.72
IL-1alpha	-0.71
Eotaxin	-0.68
IGFBP-4	-0.67
TNF-beta	-0.64
Osteoprotegerin	-0.59
TNF-alpha	-0.58
IL-5	-0.53
sgp130	-0.46

H

Proteins increased by p38 Inhibition

Factor	Log2 change from SEN(XRA)	p-value
VEGF-D	0.01	0.99
MIP-1beta	0.03	0.91
GITR	0.05	0.87
FGF-9	0.09	0.67
b-NGF	0.09	0.82
HGF	0.10	0.78
sTNF RII	0.15	0.69
Oncostatin M	0.15	0.48
Axl	0.18	0.51
IL-2 Rapha	0.21	0.37
bFGF	0.21	0.26
Angiopoietin-2	0.22	0.32
AgRP	0.31	0.40
BTC	0.34	0.55
VEGF	0.39	0.57
Amphiregulin	0.41	0.12
HCC-4	1.04	0.16

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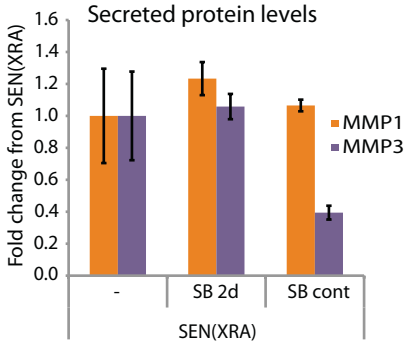

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Figure S2

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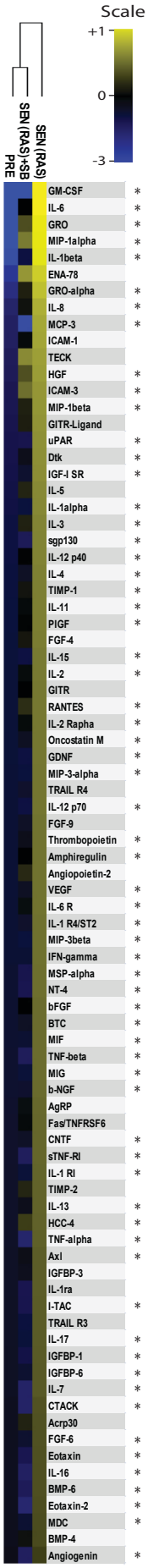

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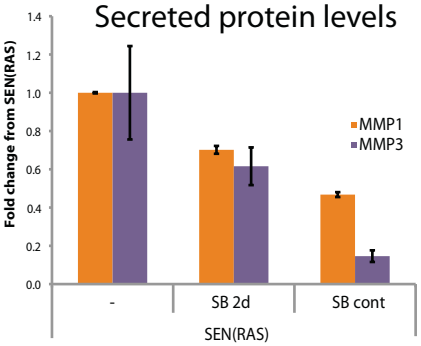

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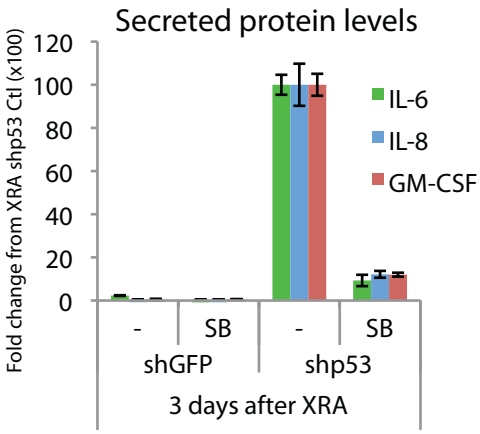

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Factor	p38MAPK-dependent in XRA and RAS
GM-CSF	•
IL-6	•
GRO	•
GRO-alpha	•
MCP-3	•
IL-8	•
IL-5	
IL-1alpha	•
IL-3	
sgp130	•
IL-2	•
GDNF	•
TNF-beta	•
MIG	•
CNTF	•
TNF-alpha	•
IGFBP-1	
IGFBP-6	•
IL-7	•
Eotaxin	•
BMP-6	
BMP-4	
Angiogenin	•

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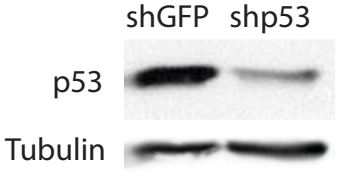

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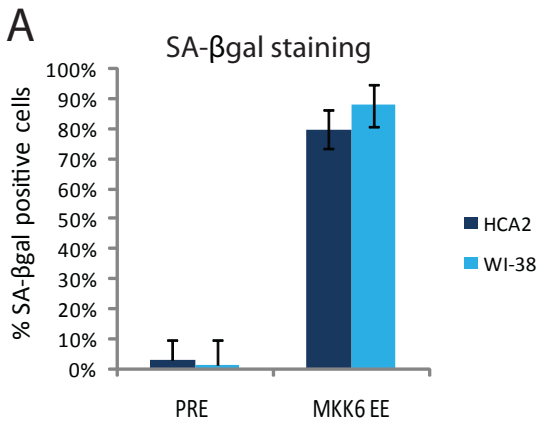
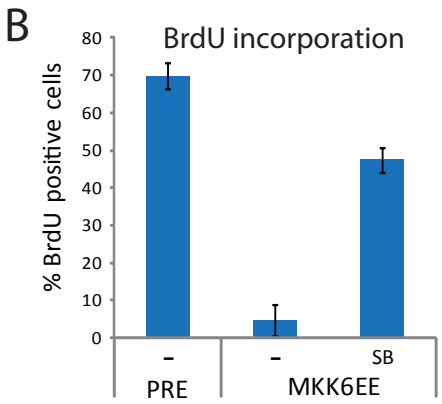
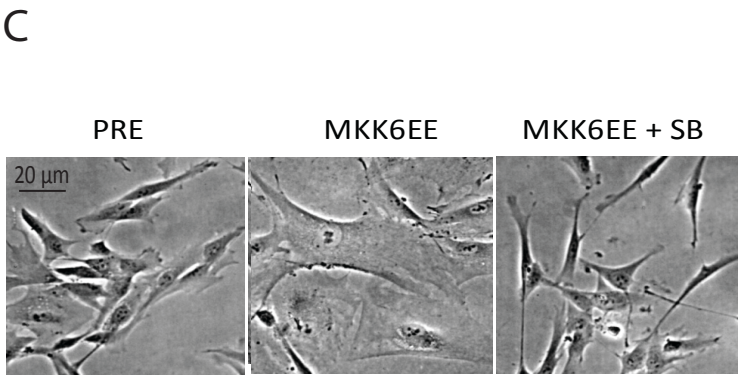
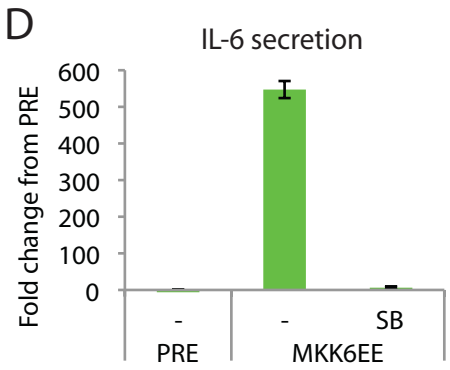
Figure S3









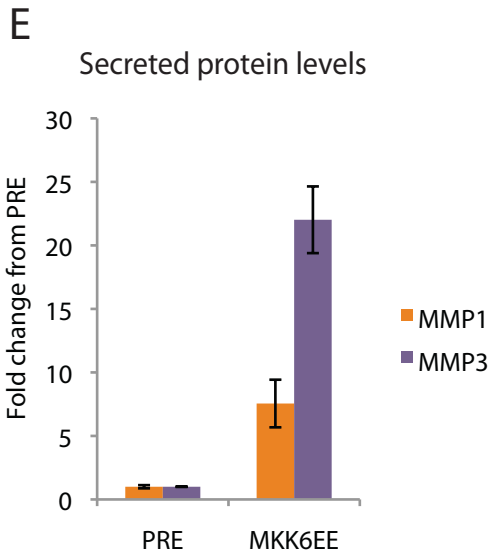


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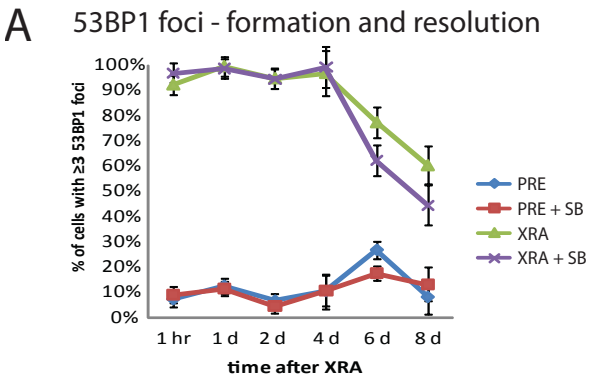
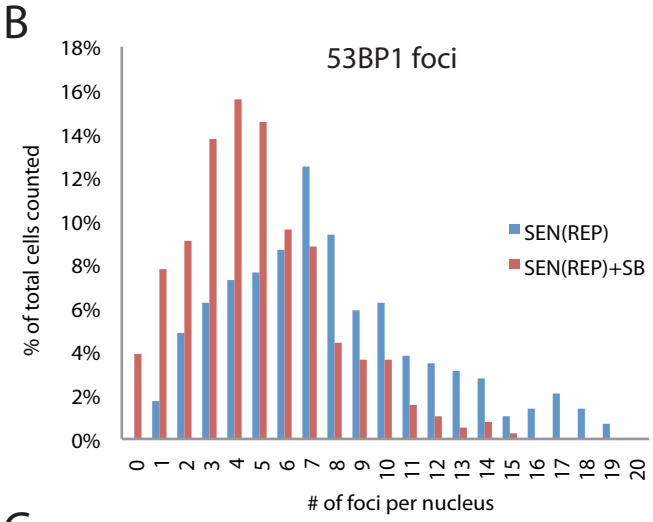
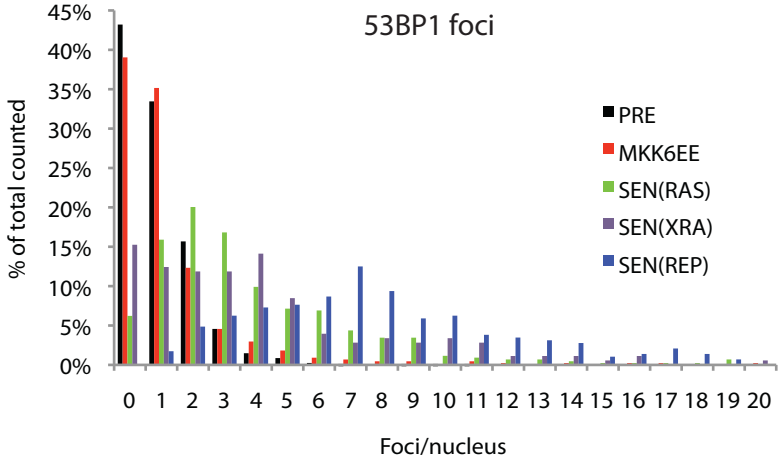
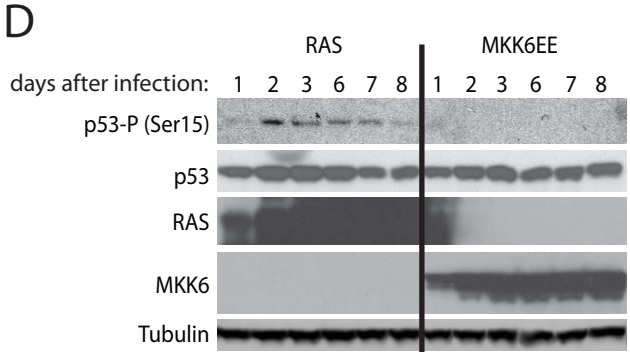
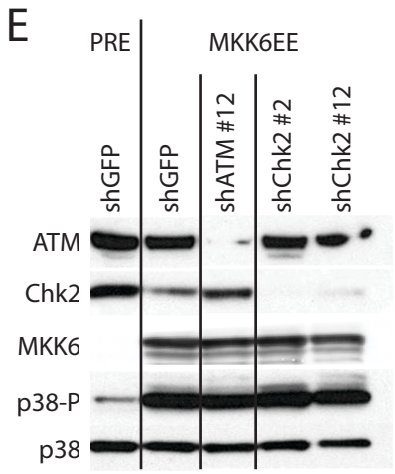
Figure S4

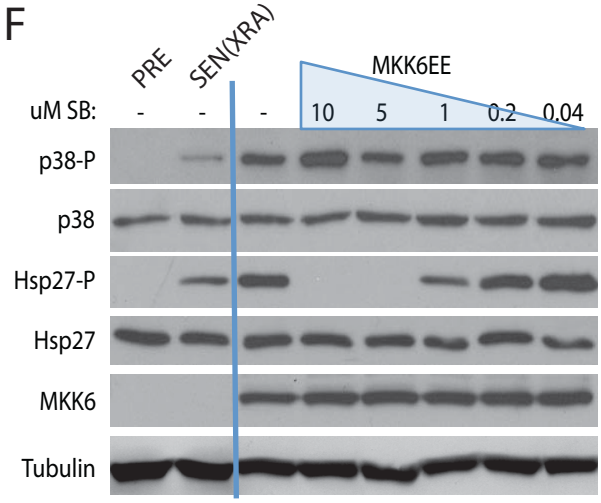












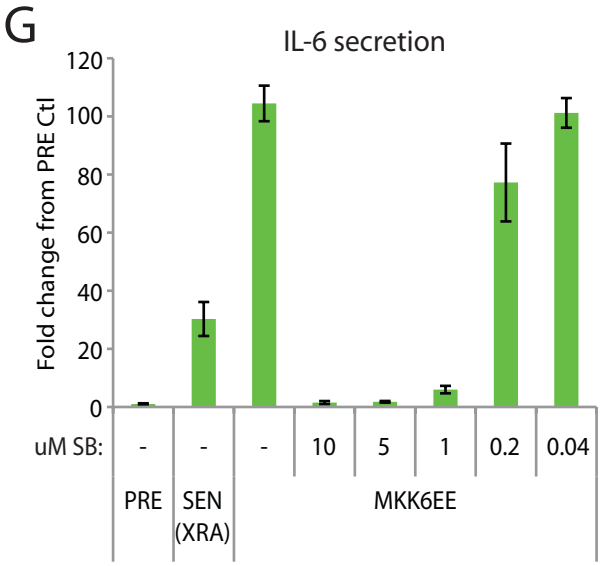


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Figure S5

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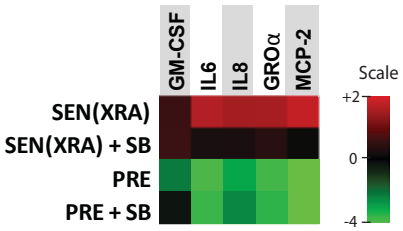

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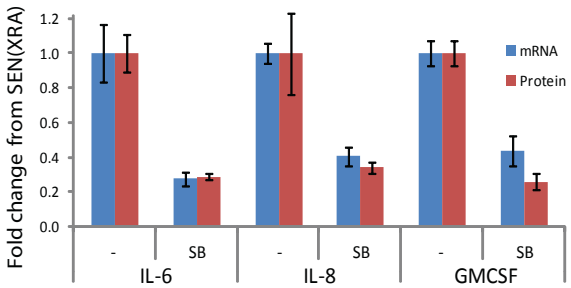

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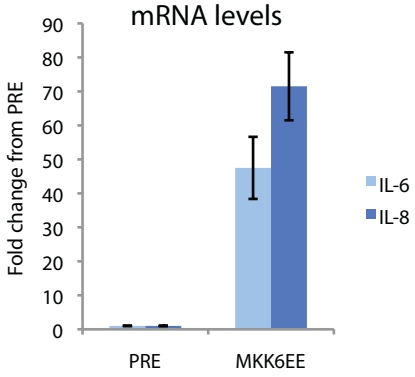

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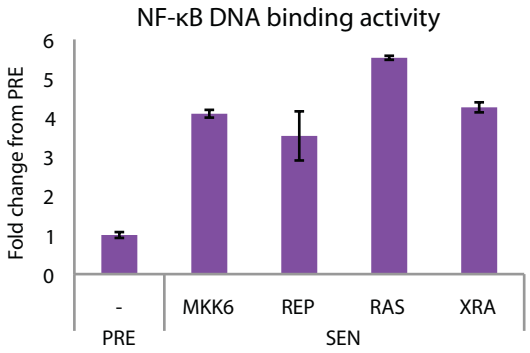

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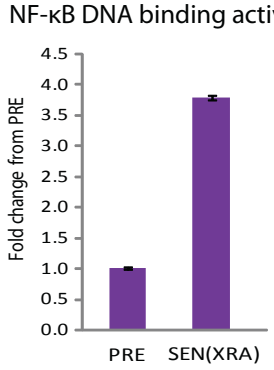

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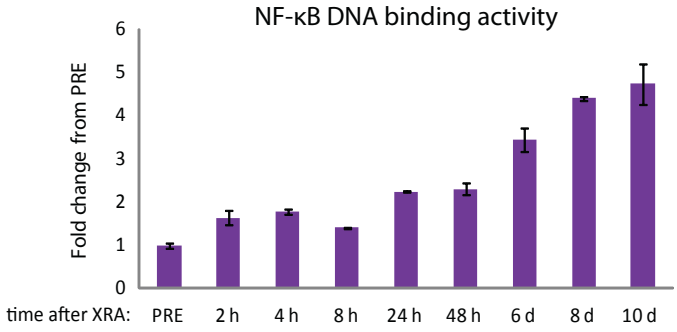

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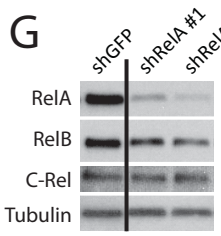

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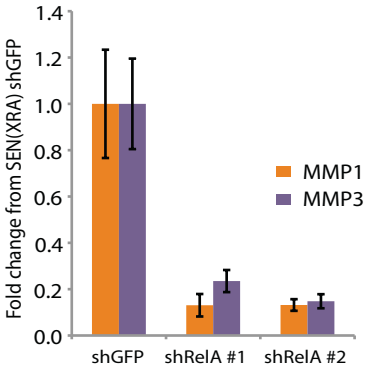

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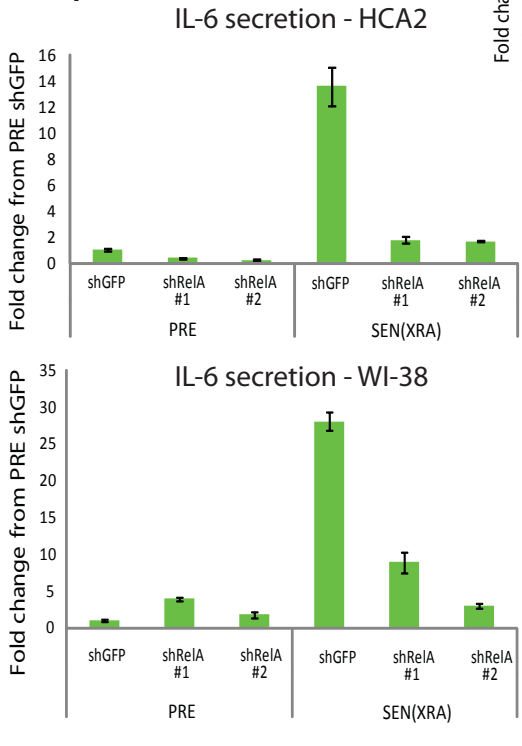

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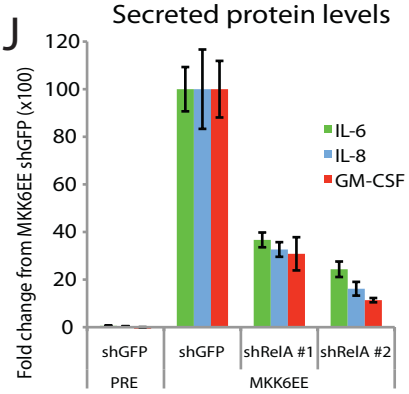

Table S1: Statistical Analyses for Figures

Figure 1C

IL-6			p-values	
Sample	Average	Standard Dev	PRE	SEN(XRA)
PRE	1.00	0.16		
SEN(XRA)	6.16	1.31	2.47E-03	
SEN(XRA)+SB	2.06	0.11	6.31E-04	5.69E-03

IL-8			p-values	
Sample	Average	Standard Dev	PRE	SEN(XRA)
PRE	1.00	0.28		
SEN(XRA)	6.39	1.50	3.65E-03	
SEN(XRA)+SB	2.34	0.32	5.63E-03	1.03E-02

GM-CSF			p-values	
Sample	Average	Standard Dev	PRE	SEN(XRA)
PRE	1.00	0.43		
SEN(XRA)	6.86	0.51	1.09E-04	
SEN(XRA)+SB	1.66	0.19	7.18E-02	7.85E-05

Figure 1D

IL-6				p-values				
				PRE			SEN(XRA)	
Sample		Average	Standard Dev	shGFP	shp38 1	shp38 2	shGFP	shp38 1
PRE	shGFP	1.00	0.10					
	shp38 #1	0.93	0.11	4.67E-01				
	shp38 #2	0.35	0.00	3.97E-04	6.85E-04			
SEN(XRA)	shGFP	7.08	0.62	7.36E-05	7.07E-05	4.67E-05		
	shp38 #1	2.88	0.56	4.51E-03	3.97E-03	1.40E-03	9.46E-04	
	shp38 #2	1.24	0.08	7.25E-02	4.18E-02	2.40E-04	1.07E-03	2.91E-02

Figure 1F

Invasion				p-values				
				PRE			SEN(XRA)	
Sample		Average	Standard Dev	shGFP	shp38 #1	shp38 #2	shGFP	shp38 #1
PRE	shGFP	50.00	8.52					
	shp38 #1	51.25	23.31	9.23E-01				
	shp38 #2	48.50	5.80	7.81E-01	8.27E-01			
SEN(XRA)	shGFP	306.75	35.87	8.54E-06	2.09E-05	7.58E-06		
	shp38 #1	136.50	27.20	9.08E-04	3.13E-03	7.28E-04	2.77E-04	
	shp38 #2	104.25	21.70	3.49E-03	1.58E-02	2.54E-03	7.05E-05	1.13E-01

Figure 2D

IL-6			p-values		
Sample	Average	Standard Dev	PRE	SEN(XRA)	SEN(RAS)
PRE	1.00	0.10			
SEN(XRA)	15.60	1.52	1.09E-04		
SEN(RAS)	85.73	17.41	1.08E-03	2.03E-03	
SEN(XRA)+GSE	438.31	20.06	2.95E-06	3.45E-06	3.87E-05

Figure 2F

IL-6				p-values		
Sample		Average	Standard Dev	L3P -	L3P+SB	GSE -
L3P	-	0.65	0.05			
	SB	0.38	0.04	1.81E-03		
GSE	-	100.00	4.58	2.99E-06	2.96E-06	
	SB	9.56	0.76	3.51E-05	3.11E-05	4.59E-06

IL-8				p-values		
Sample		Average	Standard Dev	L3P -	L3P+SB	GSE -
L3P	-	0.39	0.06			
	SB	0.42	0.09	6.39E-01		
GSE	-	100.00	2.32	1.89E-07	1.89E-07	
	SB	0.57	0.89	1.40E-01	1.31E-01	2.59E-07

GM-CSF				p-values		
Sample		Average	Standard Dev	L3P -	L3P+SB	GSE -
L3P	-	0.45	0.03			
	SB	0.50	0.03	1.48E-01		
GSE	-	100.00	9.59	5.43E-05	5.42E-05	
	SB	7.13	0.58	2.31E-05	2.25E-05	7.46E-05

Figure 4B

53BP1 foci	p-values

Sample	% ≥3 53BP1 foci	Standard Error	PRE (Vector)	MKK6EE	SEN(RAS)	SEN(XRA)
PRE (Vector)	14.1%	3.5%				
MKK6EE	18.3%	4.8%	5.12E-02			
SEN(RAS)	57.8%	4.8%	1.03E-58	2.22E-33		
SEN(XRA)	60.5%	7.5%	5.37E-41	7.29E-25	5.51E-01	
SEN(REP)	93.4%	5.9%	5.00E-130	1.87E-87	1.90E-25	1.79E-18

Figure 4D

IL-6				p-values			
				PRE	MKK6EE		
Sample		Average	Standard Dev	shGFP	shGFP	shATM #12	shChk2 #2
PRE	shGFP	0.44	0.04				
MKK6EE	shGFP	100.00	12.74	1.73E-04			
	shATM #12	116.13	4.37	1.35E-06	1.07E-01		
	shChk2 #2	91.12	18.09	9.70E-04	5.25E-01	8.04E-02	
	shChk2 #12	94.55	6.92	1.33E-05	7.72E-01	1.44E-02	5.99E-01

IL-8				p-values			
				PRE	MKK6EE		
Sample		Average	Standard Dev	shGFP	shGFP	shATM #12	shChk2 #2
PRE	shGFP	0.06	0.07				
MKK6EE	shGFP	100.00	9.93	4.90E-03			
	shATM #12	121.59	13.82	2.85E-03	1.43E-01		
	shChk2 #2	82.61	12.67	1.16E-02	2.66E-01	6.93E-02	
	shChk2 #12	94.61	13.20	4.48E-03	6.57E-01	9.55E-02	3.74E-01

GM-CSF				p-values			
				PRE	MKK6EE		
Sample		Average	Standard Dev	shGFP	shGFP	shATM #12	shChk2 #2
PRE	shGFP	0.02	0.01				
MKK6EE	shGFP	100.00	17.13	4.33E-03			
	shATM #12	120.69	1.40	8.89E-04	3.00E-01		
	shChk2 #2	120.34	31.50	3.26E-02	4.03E-01	8.73E-01	
	shChk2 #12	154.57	1.78	6.61E-05	2.37E-02	9.15E-03	2.65E-01

Figure 5A

GM-CSF			p-values		
Sample	Average	Standard Dev	PRE	PRE + SB	SEN(XRA)
PRE	1.00	0.08			
PRE + SB	0.77	0.06	2.40E-02		
SEN(XRA)	24.55	1.81	1.34E-06	4.01E-04	
SEN(XRA) + SB	10.67	2.16	5.03E-04	2.31E-02	4.31E-03

IL-6			p-values		
Sample	Average	Standard Dev	PRE	PRE + SB	SEN(XRA)
PRE	1.00	0.06			
PRE + SB	0.73	0.05	6.66E-03		
SEN(XRA)	17.00	2.81	7.71E-05	4.43E-03	
SEN(XRA) + SB	4.63	0.70	2.95E-04	1.59E-02	1.01E-02

IL-8			p-values		
Sample	Average	Standard Dev	PRE	PRE + SB	SEN(XRA)
PRE	1.00	0.04			
PRE + SB	0.90	0.04	2.94E-02		
SEN(XRA)	17.80	1.03	4.34E-07	2.06E-04	
SEN(XRA) + SB	7.20	0.98	1.29E-04	1.19E-02	1.44E-03

GROα			p-values		
Sample	Average	Standard Dev	PRE	PRE + SB	SEN(XRA)
PRE	1.00	0.15			
PRE + SB	1.03	0.11	8.19E-01		
SEN(XRA)	8.28	0.36	2.44E-07	1.15E-04	
SEN(XRA) + SB	3.64	0.02	1.79E-05	8.74E-04	4.09E-04

MCP-2			p-values		
Sample	Average	Standard Dev	PRE	PRE + SB	SEN(XRA)
PRE	1.00	0.36			
PRE + SB	0.15	0.04	3.58E-02		
SEN(XRA)	91.46	16.39	9.01E-05	4.96E-03	
SEN(XRA) + SB	15.63	2.89	3.35E-04	1.70E-02	8.61E-03

IL-1α			p-values		
Sample	Average	Standard Dev	PRE	PRE + SB	SEN(XRA)
PRE	1.00	0.26			
PRE + SB	0.15	0.05	1.92E-02		
SEN(XRA)	27.71	5.08	8.18E-04	5.35E-03	
SEN(XRA) + SB	8.27	0.95	9.01E-04	6.79E-03	1.47E-02

Figure 5D

IL-6			p-values
Sample	Average	Standard Dev	PRE
PRE	3.11	1.35	
SEN(XRA)	100.00	52.26	3.40E-03

IL-8			p-values
Sample	Average	Standard Dev	PRE
PRE	0.62	0.72	
SEN(XRA)	100.00	31.89	2.41E-04

GM-CSF			p-values
Sample	Average	Standard Dev	PRE
PRE	5.19	4.13	
SEN(XRA)	100.00	31.60	8.44E-03

Figure 5E

NF-κB transcriptional activity				p-values			
Sample		Value	Standard Dev	PRE	SEN(XRA)	SEN(XRA)+SB	SEN(XRA)+shATM
PRE	-	1.00	0.02				
SEN(XRA)	-	35.92	2.03	4.9E-07			
	SB	10.23	1.45	1.4E-07	5.1E-06		
	shATM	8.66	0.73	2.8E-07	3.3E-06	2.4E-01	
	SB + shATM	1.98	0.87	5.1E-07	6.6E-07	3.1E-07	7.5E-07

Figure 5H

IL-6				p-values				
				PRE (Vector)			SEN(RAS)	
Sample		Average	Standard Dev	shGFP	shRelA #1	shRelA #2	shGFP	shRelA #1
PRE (Vector)	shGFP	4.29	1.16					
	shRelA #1	0.57	0.04	5.04E-03				
	shRelA #2	0.18	0.02	3.51E-03	6.97E-05			
SEN(RAS)	shGFP	100.00	9.06	5.43E-05	4.52E-05	4.45E-05		
	shRelA #1	34.90	3.39	5.93E-04	3.09E-04	2.99E-04	2.62E-03	
	shRelA #2	12.17	3.75	2.55E-02	5.87E-03	5.22E-03	1.01E-04	6.38E-03

IL-8				p-values				
				PRE (Vector)			SEN(RAS)	
Sample		Average	Standard Dev	shGFP	shRelA #1	shRelA #2	shGFP	shRelA #1
PRE (Vector)	shGFP	1.20	0.55					
	shRelA #1	2.16	0.49	1.34E-01				
	shRelA #2	1.13	0.66	9.08E-01	9.77E-02			
SEN(RAS)	shGFP	100.00	6.24	2.30E-04	1.11E-05	1.07E-05		
	shRelA #1	32.67	5.73	5.19E-03	7.76E-04	6.91E-04	1.61E-04	
	shRelA #2	14.11	1.69	2.15E-03	3.02E-04	2.46E-04	2.12E-05	5.75E-03

GM-CSF				p-values				
				PRE (Vector)			SEN(RAS)	
Sample		Average	Standard Dev	shGFP	shRelA #1	shRelA #2	shGFP	shRelA #1
PRE (Vector)	shGFP	0.89	0.45					
	shRelA #1	1.27	0.42	3.49E-01				
	shRelA #2	0.79	0.46	7.97E-01	2.54E-01			
SEN(RAS)	shGFP	100.00	5.25	5.28E-06	5.35E-06	5.26E-06		
	shRelA #1	41.23	3.08	2.33E-05	2.40E-05	2.31E-05	7.48E-05	
	shRelA #2	26.51	3.41	2.09E-04	2.20E-04	2.06E-04	3.45E-05	5.16E-03

Table S2: Statistical Analyses for Supplementary Figures

Figure S1B

SA-β-galactosidase			p-values
Sample	SA-βgal positive	Standard Dev	PRE
PRE	15.8%	4.9%	
SEN(XRA)	84.7%	7.8%	1.12E-03

Figure S1D

IL-6				p-values		
Sample		Average	Standard Dev	PRE	PRE+SB	SEN(XRA)
PRE	-	1.00	0.10			
	SB	0.66	0.07	5.44E-02		
	-	60.39	1.09	1.70E-04	1.67E-04	
SEN(XRA)	SB	19.39	3.69	1.95E-02	1.88E-02	4.37E-03

Figure S1E

IL-6				p-values		
Sample - HCA2		Average	Standard Dev	PRE	PRE+SB	SEN(REP)
PRE	-	1.00	0.50			
	SB	0.45	0.10	7.32E-02		
	-	21.39	4.53	5.19E-04	4.38E-04	
SEN(REP)	SB	6.08	0.30	2.12E-05	1.34E-06	7.79E-03

IL-6				p-values		
Sample - WI-38		Average	Standard Dev	PRE	PRE+SB	SEN(REP)
PRE	-	1.00	0.42			
	SB	0.67	0.13	1.85E-01		
	-	298.94	60.80	6.50E-05	6.46E-05	
SEN(REP)	SB	136.33	47.51	1.26E-03	1.25E-03	5.59E-03

Figure S1I

MMP-1				p-values	
Sample		Average	Standard Dev	-	SB 2d
SEN(XRA)	-	1.00	0.30		
	SB 2d	1.23	0.10	2.66E-01	
	SB cont	1.07	0.04	7.24E-01	5.67E-02

MMP-3				p-values	
Sample		Average	Standard Dev	-	SB 2d
SEN(XRA)	-	1.00	0.28		
	SB 2d	1.06	0.08	7.44E-01	
	SB cont	0.39	0.04	2.02E-02	2.18E-04

Figure S2B

MMP-1				p-values	
Sample		Average	Standard Dev	-	SB 2d
SEN(RAS)	-	1.00	0.00		
	SB 2d	0.70	0.02	2.42E-03	
	SB cont	0.47	0.01	3.11E-04	5.30E-03

MMP-3				p-values	
Sample		Average	Standard Dev	-	SB 2d
SEN(RAS)	-	1.00	0.24		
	SB 2d	0.62	0.10	8.10E-02	
	SB cont	0.15	0.03	7.28E-03	1.40E-03

Figure S2E

IL-6				p-values		
Sample		Average	Standard Dev	shGFP	shGFP+SB	shp53
shGFP	-	2.31	0.16			
	SB	0.39	0.13	8.45E-05		
shp53	-	100.00	4.63	3.35E-06	3.09E-06	
	SB	9.31	2.63	1.52E-02	7.70E-03	1.51E-04

IL-8				p-values		
Sample		Average	Standard Dev	shGFP	shGFP+SB	shp53
shGFP	-	0.46	0.10			
	SB	0.44	0.00	8.16E-01		
shp53	-	100.00	9.76	6.04E-05	6.04E-05	
	SB	12.16	1.58	2.15E-04	2.13E-04	1.04E-04

GM-CSF				p-values		
Sample		Average	Standard Dev	shGFP	shGFP+SB	shp53
shGFP	-	0.55	0.27			
	SB	0.63	0.05	6.50E-01		
shp53	-	100.00	5.09	4.56E-06	4.55E-06	
	SB	11.99	0.91	3.16E-05	2.77E-05	7.87E-06

Figure S3A

SA-β-galactosidase				p-values
Cell Type	Sample	SA-βgal positive	Standard Dev	PRE (Vector)
HCA2	PRE (Vector)	3.2%	6.3%	
	MKK6EE	80.0%	6.3%	5.76E-68
WI-38	PRE (Vector)	1.5%	7.1%	
	MKK6EE	88.0%	6.3%	2.65E-74

Figure S3B

BrdU Quantification			p-values	
Sample	% BrdU positive	Standard Error	PRE (Vector)	MKK6EE
PRE	69.9%	3.4%		
MKK6EE	4.8%	4.2%	3.02E-129	
MKK6EE + SB	47.4%	3.2%	2.44E-22	3.15E-67

Figure S3D

IL-6			p-values	
Sample	Average	Standard Dev	PRE (Vector)	MKK6EE
PRE	1.00	0.13		
MKK6EE	547.22	23.32	2.20E-06	
MKK6EE + SB	6.23	3.57	6.45E-02	2.40E-06

Figure S3E

MMP-1				p-values
Sample		Average	Standard Dev	PRE
SEN(RAS)	-	1.00	0.13	
	SB 2d	7.55	1.87	3.79E-03

MMP-3				p-values
Sample		Average	Standard Dev	PRE
SEN(RAS)	-	1.00	0.01	
	SB 2d	22.02	2.63	1.57E-04

Figure S4A

53BP1 foci				p-values
1 hour		% ≥3 53BP1 foci	Standard Error	XRA
XRA	-	96.5%	4.4%	
	SB	97.0%	3.8%	6.02E-01

53BP1 foci				p-values
1 day		% ≥3 53BP1 foci	Standard Error	XRA
XRA	-	99.6%	3.9%	
	SB	98.8%	3.9%	1.30E-01

53BP1 foci				p-values
2 days		% ≥3 53BP1 foci	Standard Error	XRA
XRA	-	94.8%	4.0%	
	SB	94.6%	4.0%	9.14E-01

53BP1 foci				p-values
4 days		% ≥3 53BP1 foci	Standard Error	XRA
XRA	-	96.9%	8.8%	
	SB	99.3%	8.3%	1.35E-01

53BP1 foci				p-values
6 days		% ≥3 53BP1 foci	Standard Error	XRA
XRA	-	77.4%	6.0%	
	SB	62.3%	6.1%	1.15E-04

53BP1 foci				p-values
8 days		% ≥3 53BP1 foci	Standard Error	XRA
XRA	-	60.5%	7.5%	
	SB	44.6%	8.0%	3.75E-03

Figure S4G

IL-6				p-values						
				PRE	SEN(XRA)	MKK6EE				
Sample	uM SB	Average	Standard Dev	-	-	-	10	5	1	0.2
PRE	-	1.00	0.28							
SEN(XRA)	-	30.28	5.85	9.78E-04						
MKK6EE	-	104.46	6.12	8.15E-06	1.10E-04					
	10	1.51	0.53	2.16E-01	1.06E-03	8.40E-06				
	5	1.77	0.30	3.04E-02	1.08E-03	8.40E-06	4.98E-01			
	1	5.98	1.28	2.76E-03	2.16E-03	1.08E-05	5.07E-03	5.20E-03		
	0.2	77.26	13.40	5.94E-04	5.10E-03	3.30E-02	6.11E-04	6.18E-04	7.84E-04	
	0.04	101.20	5.10	4.48E-06	9.32E-05	5.17E-01	4.65E-06	4.63E-06	6.17E-06	4.45E-02

Figure S5A

GM-CSF			p-values		
Sample	Average	Standard Dev	PRE	PRE + SB	SEN(XRA)
PRE	1.00	0.62			
PRE + SB	3.21	0.17	9.21E-03		
SEN(XRA)	5.31	1.92	5.24E-03	2.19E-01	
SEN(XRA) + SB	5.37	0.96	2.14E-03	8.78E-02	9.69E-01

IL-6			p-values		
Sample	Average	Standard Dev	PRE	PRE + SB	SEN(XRA)
PRE	1.00	0.09			
PRE + SB	1.21	0.01	4.13E-02		
SEN(XRA)	27.48	2.44	6.24E-07	1.36E-04	
SEN(XRA) + SB	11.70	0.56	1.90E-06	1.44E-03	1.02E-03

IL-8			p-values		
Sample	Average	Standard Dev	PRE	PRE + SB	SEN(XRA)
PRE	1.00	0.08			
PRE + SB	1.41	0.02	2.60E-03		
SEN(XRA)	15.11	1.52	1.60E-06	2.76E-04	
SEN(XRA) + SB	7.04	0.33	2.72E-06	1.76E-03	2.18E-03

GROα			p-values		
Sample	Average	Standard Dev	PRE	PRE + SB	SEN(XRA)
PRE	1.00	0.09			
PRE + SB	1.20	0.03	3.87E-02		
SEN(XRA)	23.99	1.55	9.86E-08	4.01E-05	
SEN(XRA) + SB	12.18	0.63	2.35E-06	1.64E-03	5.88E-04

MCP-2			p-values		
Sample	Average	Standard Dev	PRE	PRE + SB	SEN(XRA)
PRE	1.00	0.14			
PRE + SB	0.90	0.02	4.28E-01		
SEN(XRA)	145.65	29.92	4.46E-04	2.97E-03	
SEN(XRA) + SB	53.45	5.62	3.94E-04	5.68E-03	1.50E-02

Figure S5B

Factor	Sample	mRNA level	Protein level	mRNA StDev	Protein StDev	p-value mRNA vs Protein
GM-CSF	SEN(XRA)	1.00	1.00	0.07	0.07	-
	SEN(XRA)+SB	0.43	0.26	0.09	0.04	0.19
IL-6	SEN(XRA)	1.00	1.00	0.17	0.11	-
	SEN(XRA)+SB	0.27	0.29	0.04	0.02	0.05
IL-8	SEN(XRA)	1.00	1.00	0.06	0.07	-
	SEN(XRA)+SB	0.40	0.34	0.06	0.04	0.72

Figure S5C

IL-6			p-values
Sample	Average	Standard Dev	PRE
PRE	1.00	0.06	
MKK6EE	47.50	9.14	1.88E-02

IL-8			p-values
Sample	Average	Standard Dev	PRE
PRE	1.00	0.00	
MKK6EE	71.51	1.05	1.11E-04

Figure S5D

NF-kB DNA Binding			
Sample	Value	StDev	p-value vs PRE
PRE	1.00	0.075	-
SEN(MKK6)	4.12	0.099	5.28E-04
SEN(REP)	3.53	0.628	2.95E-02
SEN(RAS)	5.53	0.052	2.01E-04
SEN(XRA)	4.26	0.128	1.66E-06

Figure S5E

NF-kB DNA Binding			p-values
Sample - WI-38	Average	Standard Dev	PRE
PRE	1.00	0.02	-
SEN(XRA)	3.78	0.03	8.46E-05

Figure S5F

NF-kB DNA Binding			
Time after XRA	Value	StDev	p-value vs PRE
PRE	1.00	0.06	-
2 hr	1.64	0.18	4.13E-02
4 hr	1.77	0.06	6.66E-03
8 hr	1.40	0.01	1.27E-02
24 hr	2.25	0.03	1.60E-03

48 hr	2.31	0.15	7.35E-03
6 day	3.44	0.27	6.46E-03
8 day	4.39	0.04	2.58E-04
10 day	4.73	0.48	8.28E-03

Figure S5H

MMP-1				p-values	
Sample		Average	Standard Dev	shGFP	shRelA #1
SEN(XRA)	shGFP	1.00	0.23		
	shRelA #1	0.13	0.05	6.58E-03	
	shRelA #2	0.13	0.03	6.07E-03	9.75E-01

MMP-3				p-values	
Sample		Average	Standard Dev	shGFP	shRelA #1
SEN(XRA)	shGFP	1.00	0.14		
	shRelA #1	0.23	0.05	8.02E-04	
	shRelA #2	0.15	0.03	4.68E-04	5.60E-02

Figure S5I

IL-6				p-values				
				PRE			SEN(XRA)	
Sample - HCA2		Average	Standard Dev	shGFP	shRelA #1	shRelA #2	shGFP	shRelA #1
PRE	shGFP	1.00	0.09					
	shRelA #1	0.38	0.05	4.89E-04				
	shRelA #2	0.25	0.01	1.34E-04	1.33E-02			
SEN(XRA)	shGFP	13.61	1.49	1.25E-04	1.03E-04	9.92E-05		
	shRelA #1	1.79	0.28	9.16E-03	9.80E-04	6.53E-04	1.72E-04	
	shRelA #2	1.69	0.09	7.07E-04	2.70E-05	1.09E-05	1.57E-04	5.73E-01

IL-6				p-values				
				PRE			SEN(XRA)	
Sample - WI-38		Average	Standard Dev	shGFP	shRelA #1	shRelA #2	shGFP	shRelA #1
PRE	shGFP	1.00	0.16					
	shRelA #1	3.91	0.22	4.09E-04				
	shRelA #2	1.75	0.41	5.40E-02	2.23E-02			
SEN(XRA)	shGFP	28.04	1.22	2.83E-06	1.20E-04	9.83E-05		
	shRelA #1	8.89	1.41	6.49E-04	1.81E-02	6.92E-03	5.85E-05	
	shRelA #2	3.04	0.32	5.93E-04	4.62E-02	2.80E-02	4.28E-06	2.18E-03

Figure S5J

IL-6				p-values		
				PRE (Vector)	MKK6EE	
Sample		Average	Standard Dev	shGFP	shGFP	shRelA #1
PRE (Vector)	shGFP	0.58	0.08			
MKK6EE	shGFP	100.00	9.30	2.62E-04		
	shRelA #1	36.68	3.11	3.61E-05	1.35E-03	
	shRelA #2	24.34	3.26	2.26E-04	8.19E-04	8.98E-03

IL-8				p-values		
				PRE (Vector)	MKK6EE	
Sample		Average	Standard Dev	shGFP	shGFP	shRelA #1
PRE (Vector)	shGFP	0.40	0.04			
MKK6EE	shGFP	100.00	16.67	4.06E-03		
	shRelA #1	32.65	3.07	7.76E-04	2.34E-03	
	shRelA #2	16.15	2.89	5.27E-03	1.01E-03	2.48E-03

GM-CSF				p-values		
				PRE (Vector)	MKK6EE	
Sample		Average	Standard Dev	shGFP	shGFP	shRelA #1
PRE (Vector)	shGFP	0.00	0.00			
MKK6EE	shGFP	100.00	11.88	1.49E-03		
	shRelA #1	30.83	6.98	9.59E-03	9.64E-04	
	shRelA #2	11.36	0.90	4.54E-04	2.09E-04	8.69E-03