Fig. S1

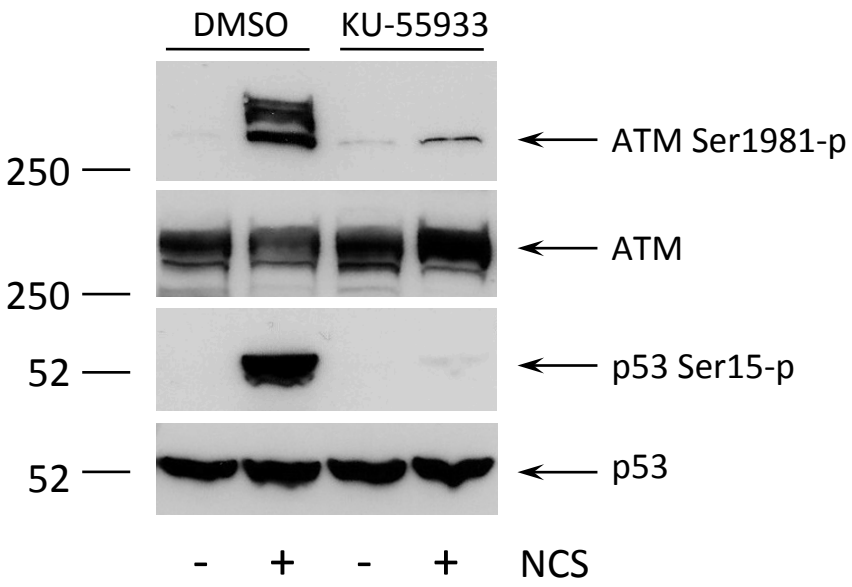
DMSO
KU-55933
250
250
52
52
ATM Ser1981-p
ATM
p53 Ser15-p
p53
-
+
-
+
NCS

Fig. S2

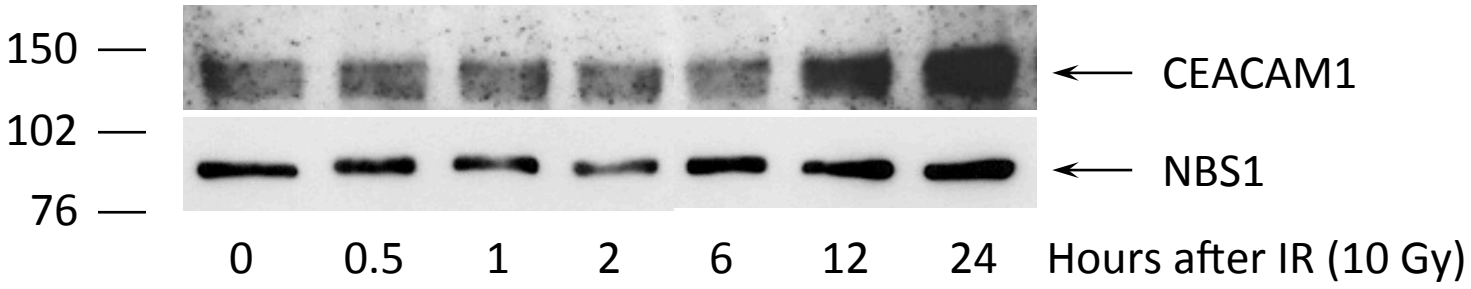

Fig. S3

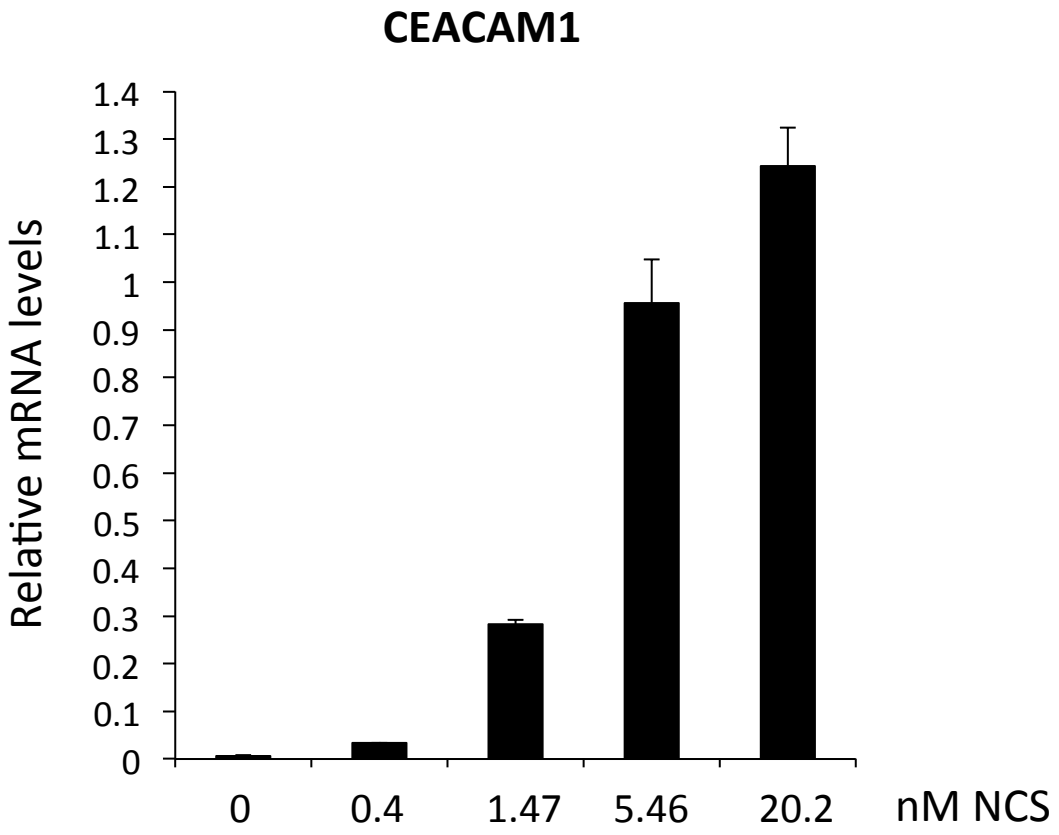
CEACAM1
Relative mRNA levels
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.4
1.47
5.46
20.2
nM NCS

Fig. S4

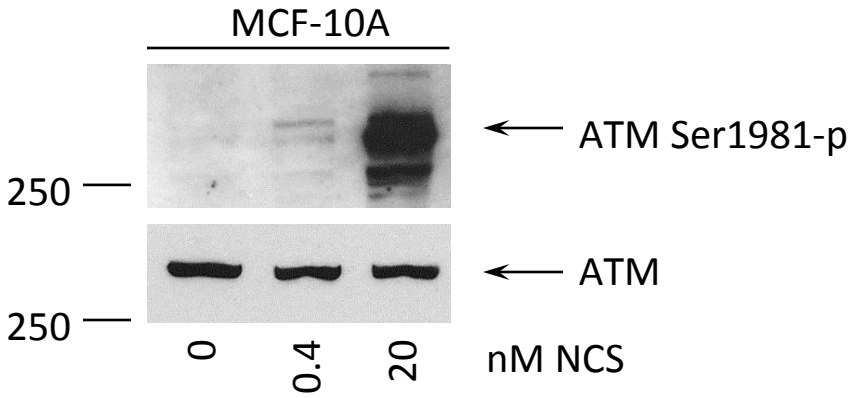

Fig. S5

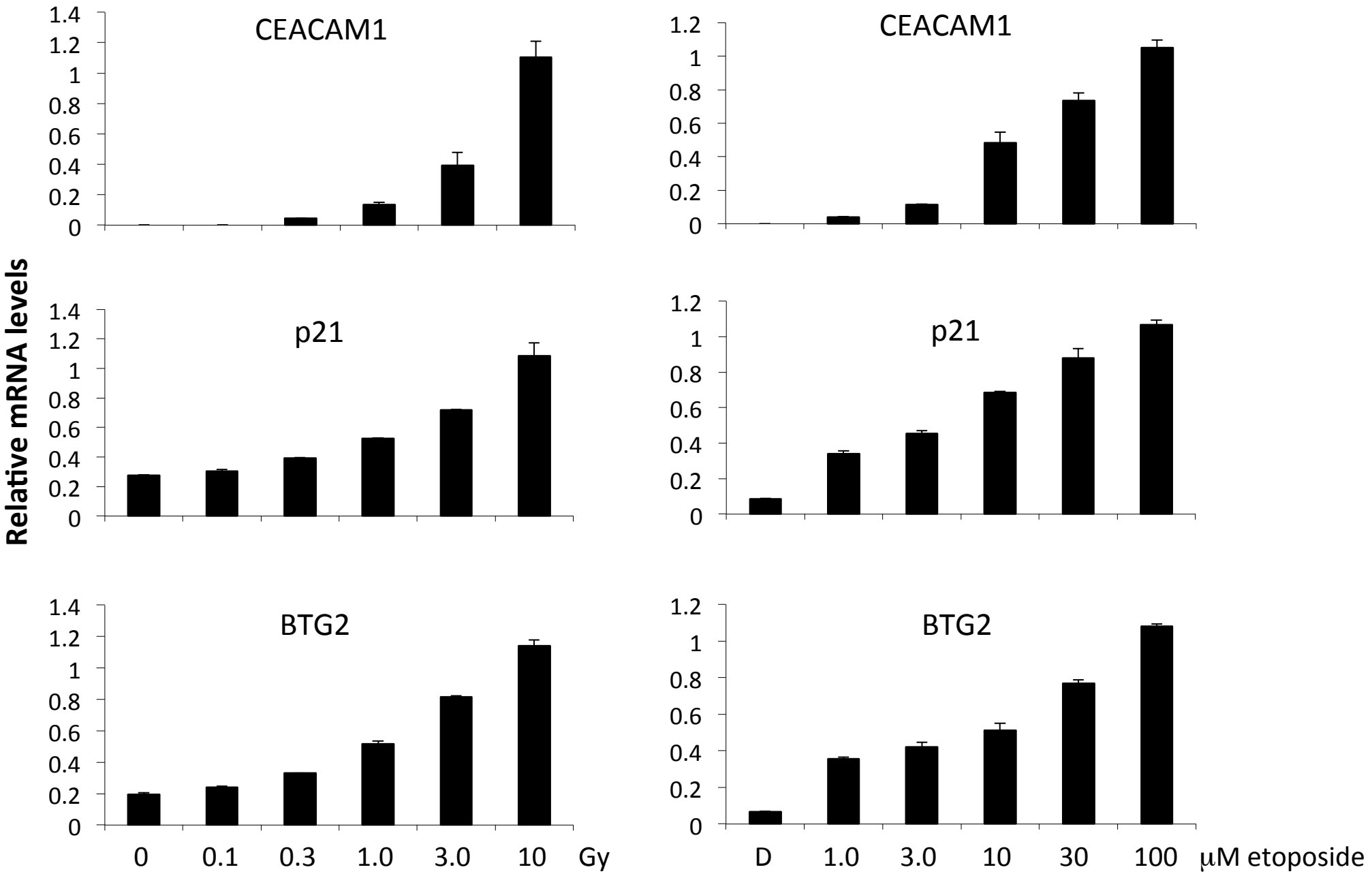
CEACAM1
p21
BTG2
Relative mRNA levels
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0 0.1 0.3 1.0 3.0 10 Gy
D 1.0 3.0 10 30 100 μM etoposide

Fig. S6

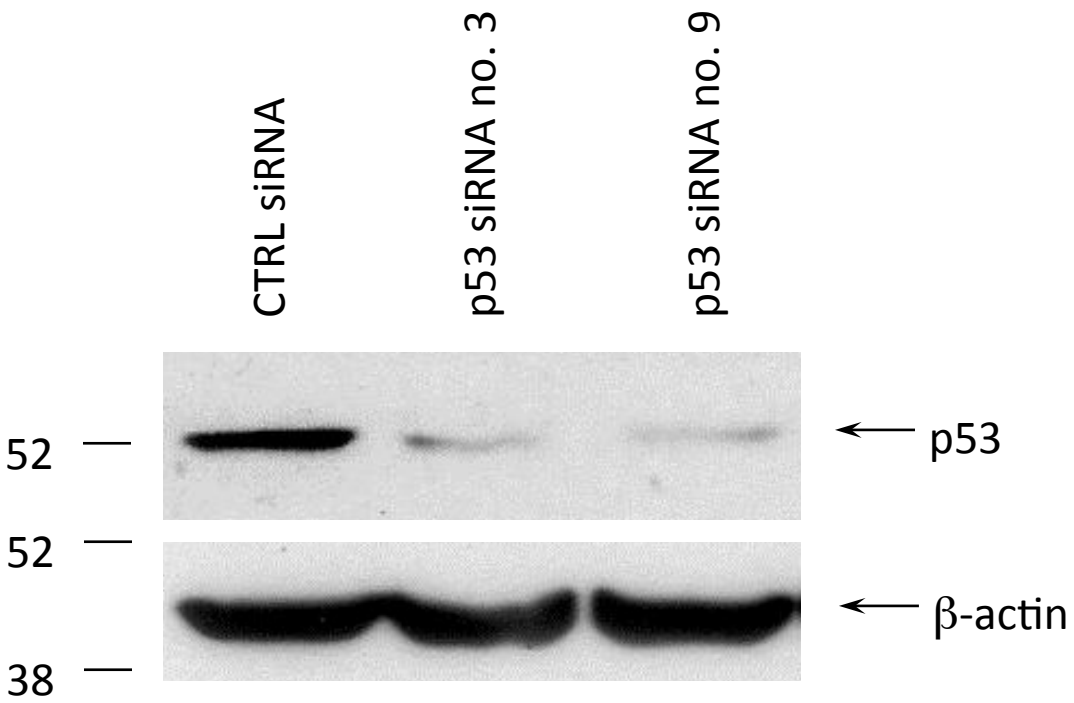

Fig. S7

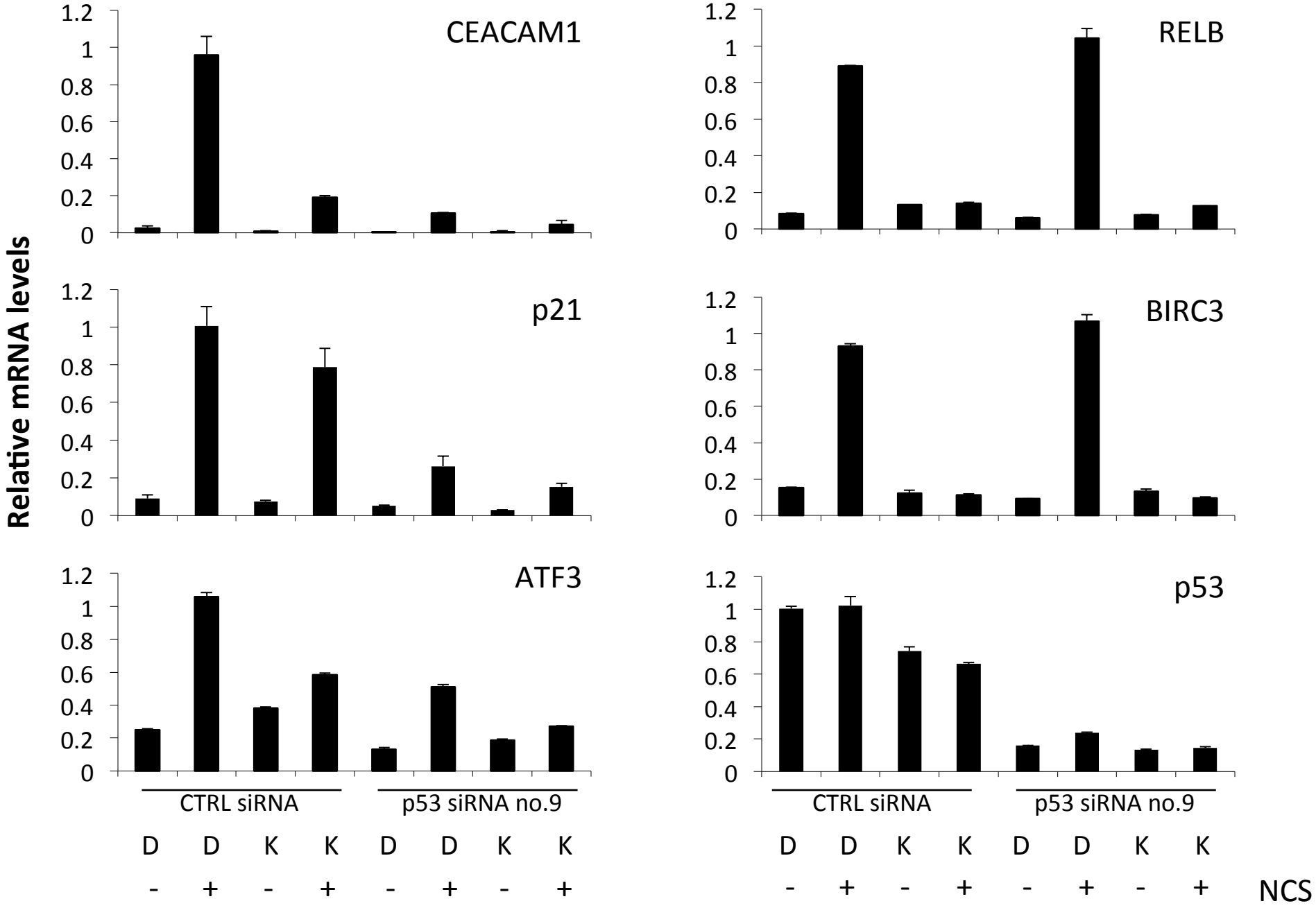
Relative mRNA levels
CEACAM1
RELB
p21
BIRC3
ATF3
p53
1.2
1
0.8
0.6
0.4
0.2
0
CTRL siRNA
p53 siRNA no.9
D D K K D D K K
- + - + - + - +
NCS

Fig. S8

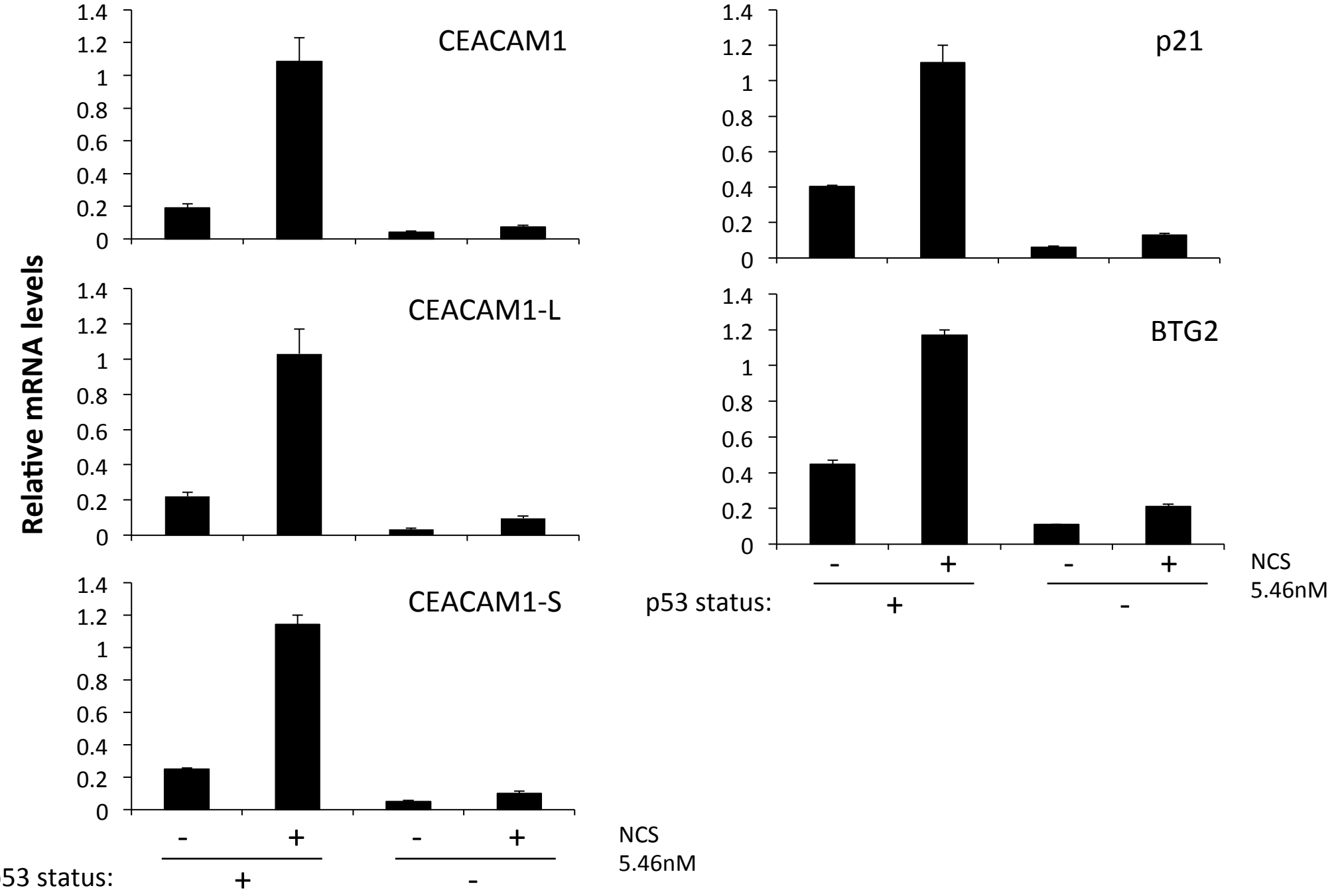
CEACAM1
CEACAM1-L
CEACAM1-S
p21
BTG2
Relative mRNA levels
1.4
1.2
1
0.8
0.6
0.4
0.2
0
- + - +
p53 status: + -
NCS
5.46nM

Fig. S9

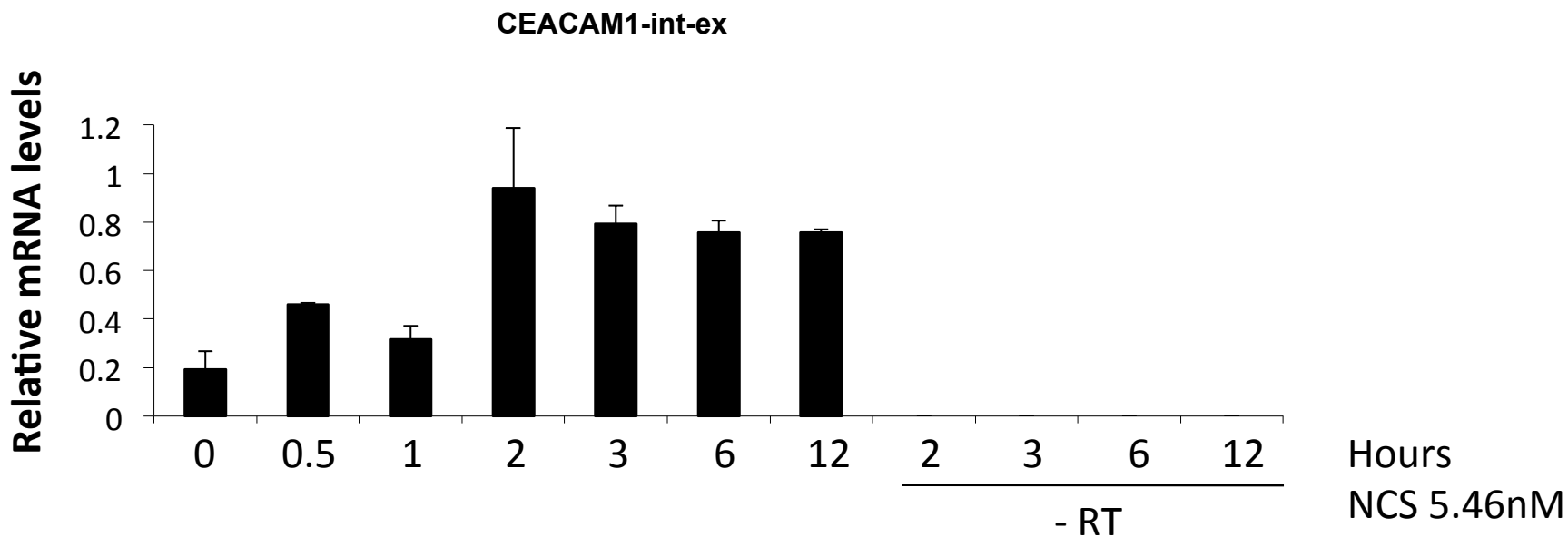
CEACAM1-int-ex
Relative mRNA levels
1.2
1
0.8
0.6
0.4
0.2
0
0
0.5
1
2
3
6
12
2
3
6
12
- RT
Hours
NCS 5.46nM

Fig. S10

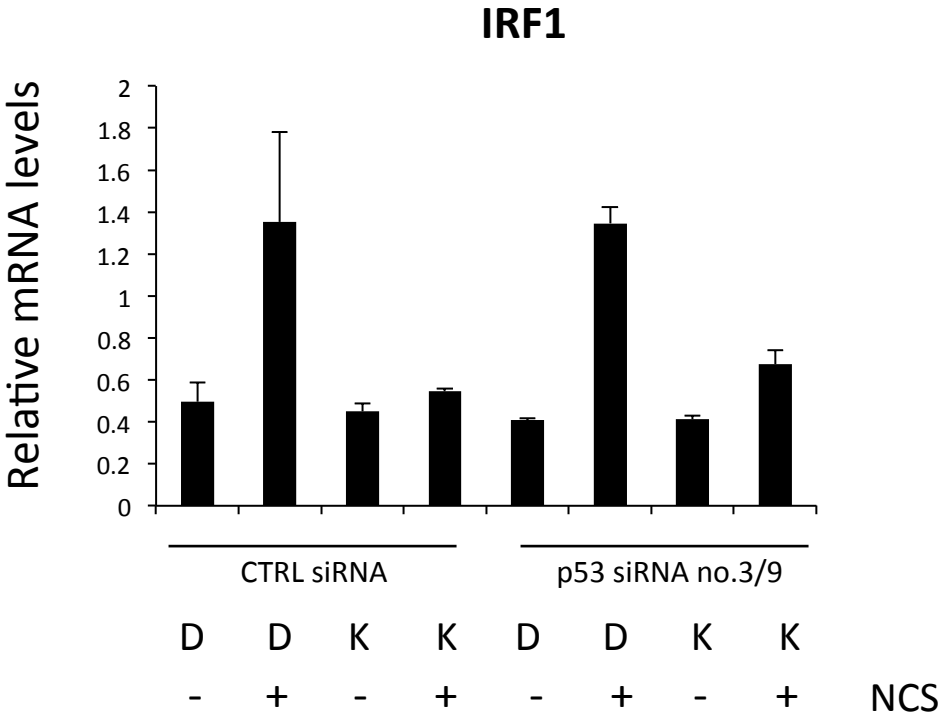
IRF1
Relative mRNA levels
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
CTRL siRNA
p53 siRNA no.3/9
D D K K D D K K
- + - + - + - + NCS

Fig. S1. KU-55933 inhibits ATM substrate phosphorylation in neocarzinostatin (NCS) treated MCF-10A cells. Parental MCF-10A cells were incubated for 1h in the presence of 10μM KU-55933 or the same dilution of DMSO (solvent) as a control, followed by 30min incubation in the presence or absence of NCS 5.46nM. At the end of the incubation, total proteins were extracted and analyzed for the levels of ATM Ser1981-p, ATM, p53 Ser15-p, or p53 expression by Western Blotting. Numbers on the left indicate kDa.

Fig. S2. Time-dependent induction of CEACAM1 expression in irradiated MCF-10A cells. MCF-10A cells were irradiated with 10 Gy X-Rays and analysed for CEACAM1 or NBS1 protein expression levels by Western Blotting after the indicated time points.

Fig. S3. **Dose-dependent increase of CEACAM1 mRNA expression after treatment with NCS.** MCF10A cells were exposed to the indicated concentrations of NCS for 4h. At the end of the incubation, total mRNA was analyzed for CEACAM1 mRNA levels by quantitative real-time PCR in triplicate. Error bars indicate SD within the internal replicates. This experiment is independent of the one presented in Fig. 1E.

Fig. S4. Phosphorylation of the ATM kinase in response to NCS. MCF-10A cells were incubated for 30min in the presence of the indicated concentrations of NCS. The cells were then lysed in the presence of phosphatase inhibitors and analyzed for the levels of ATM Ser1981-p or ATM by Western Blotting.

Fig. S5. Relative expression of CEACAM1, p21/Waf1 and BTG2 in response to X-Rays or etoposide. MCF-10A cells were incubated for 6h at 37°C after irradiation with the indicated doses of X-rays (left) or after treatment with the indicated concentrations of etoposide, or the same volume of DMSO (D) (solvent) as a control (right). At the end of the incubation total RNAs were purified and analyzed for the levels of the mRNAs of interest by quantitative real-time PCR in triplicate. Error bars indicate SD within the internal replicates.

Fig. S6. Down-regulation of p53 expression by siRNA transfection. MCF-10A cells were transfected with p53 siRNAs no. 3 or 9 as indicated, or with a scrambled siRNA (CTRL) as a control. Two days later the cells were lysed and analyzed for the levels of p53 or β-actin by Western Blotting.

Fig. S7. Induction of CEACAM1, p21/Waf1, ATF3, RELB or BIRC3 by NCS is inhibited by p53 siRNA or KU-55933. MCF-10A cells were transfected with p53 siRNA no.9 or a control (CTRL) siRNA as indicated. After 2 days, 10μM KU-55933 (K) or DMSO (D) (solvent) was added. After 1h incubation the cells were incubated in the presence or absence of 20nM NCS as indicated for an additional 4 hours. Total RNAs were then purified and analyzed for the levels of the indicated genes by quantitative real-time PCR in triplicate. Error bars indicate SD within the internal replicates.

Fig.S8. Response to NCS of CEACAM1 long (-L) or short (-S) mRNA expression as a function of the p53 status in HCT116 cells. HCT116 cells with wild type or inactivated p53 were incubated for 16h in the presence of NCS 5.46nM. At the end of the incubation total RNAs were purified and analyzed for the levels of the indicated mRNAs by quantitative real-time PCR in triplicate. Error bars indicate SD within the internal replicates.

Fig. S9. CEACAM1 primary transcript is upregulated by NCS in a time-dependent manner. Reiteration of the experiment shown in Fig. 1D, showing, in addition, that CEACAM1 primary transcript amplification does not occur if reverse transcriptase (RT) is omitted, thus demonstrating that we were not amplifying genomic DNA.

Fig. S10. NCS induces IRF1 mRNA expression in an ATM-dependent and p53-independent manner. MCF-10A cells were transfected with p53 siRNA no.3 or p53 siRNA no. 9, or a control (CTRL) siRNA as indicated, in two different experiments. Two days later, 10μM KU-55933 (K) or DMSO (D) (solvent) was added. After 1h incubation the cells were incubated in the presence or absence of 20nM NCS as indicated for an additional 4 hours. At the end of the incubation total RNAs were purified and analyzed for the levels of IRF1 by quantitative real-time PCR. Erros bars indicate SD.