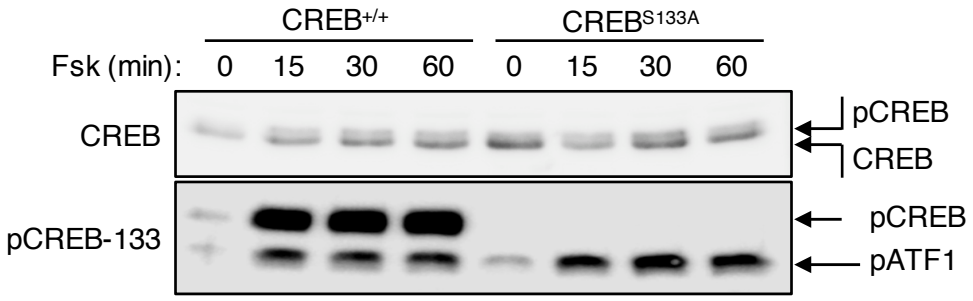
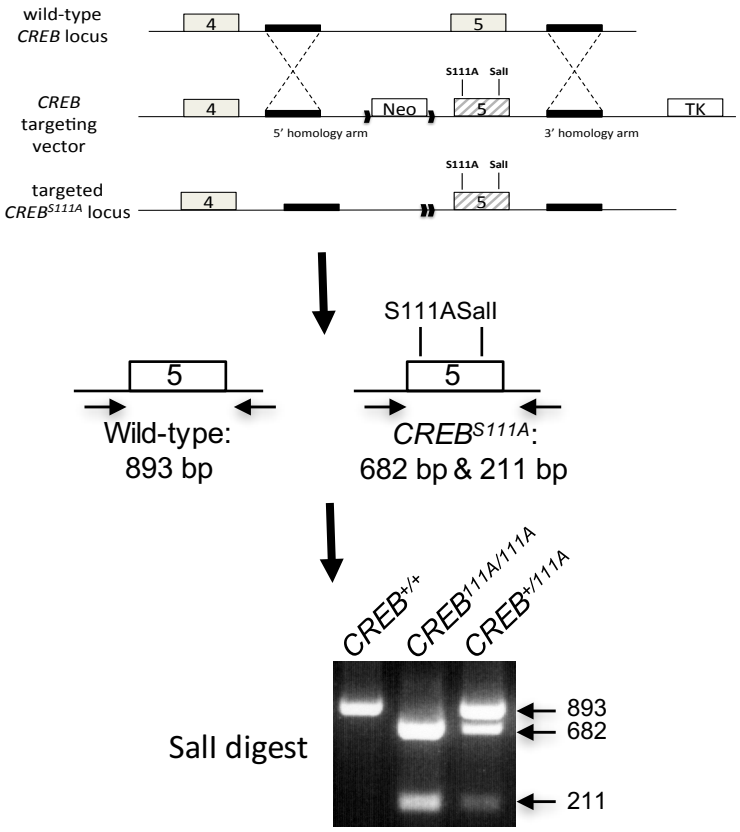
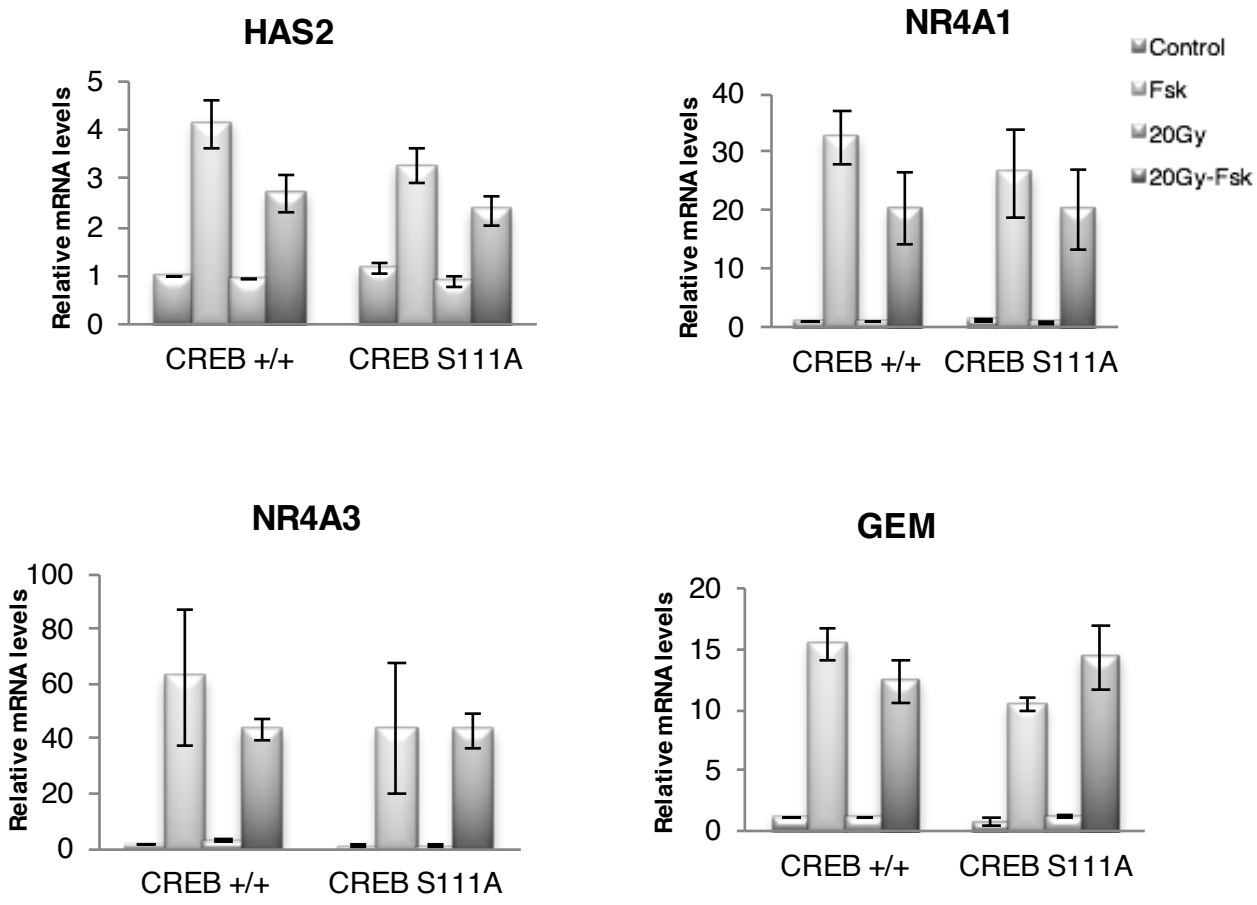
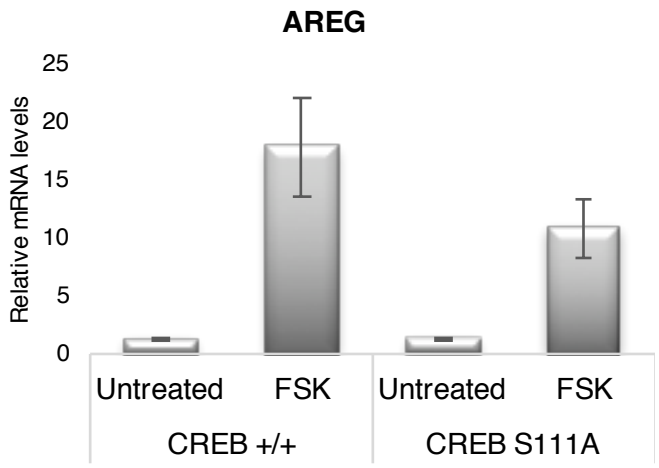

CREB+/+
CREBS133A
Fsk (min):
0
15
30
60
0
15
30
60
CREB
pCREB
CREB
pCREB-133
pCREB
pATF1


wild-type
CREB locus
4
5
S111A
SalI
CREB
targeting
vector
Neo
TK
5' homology arm
3' homology arm
targeted
CREBS111A locus
S111ASalI
Wild-type:
893 bp
CREBS111A:
682 bp & 211 bp
CREB+/+
CREB111A/111A
CREB+/111A
SalI digest
893
682
211

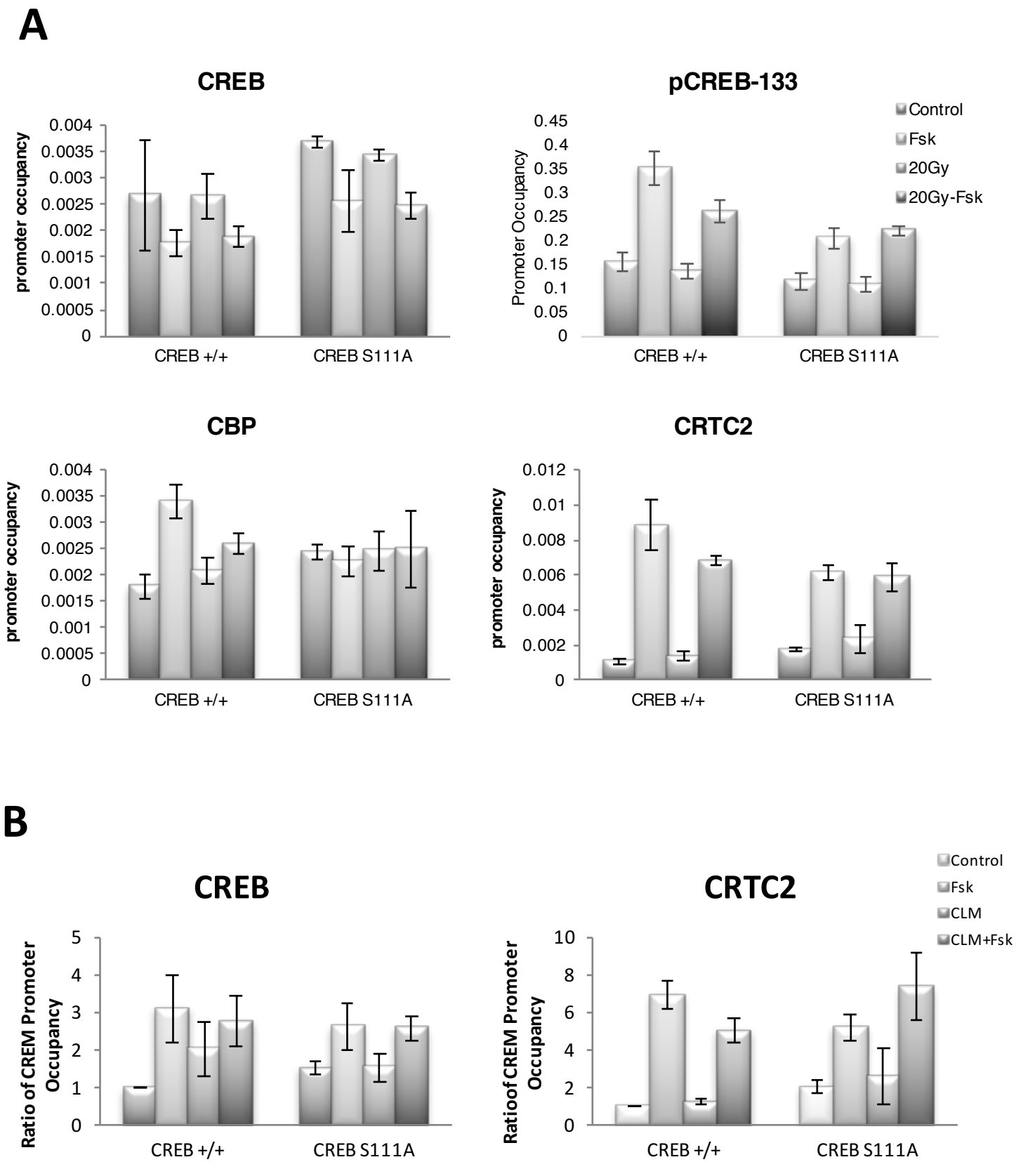

HAS2
Relative mRNA levels
0
1
2
3
4
5
CREB +/+
CREB S111A
NR4A1
Relative mRNA levels
0
10
20
30
40
CREB +/+
CREB S111A
Control
Fsk
20Gy
20Gy-Fsk
NR4A3
Relative mRNA levels
0
20
40
60
80
100
CREB +/+
CREB S111A
GEM
Relative mRNA levels
0
5
10
15
20
CREB +/+
CREB S111A

A

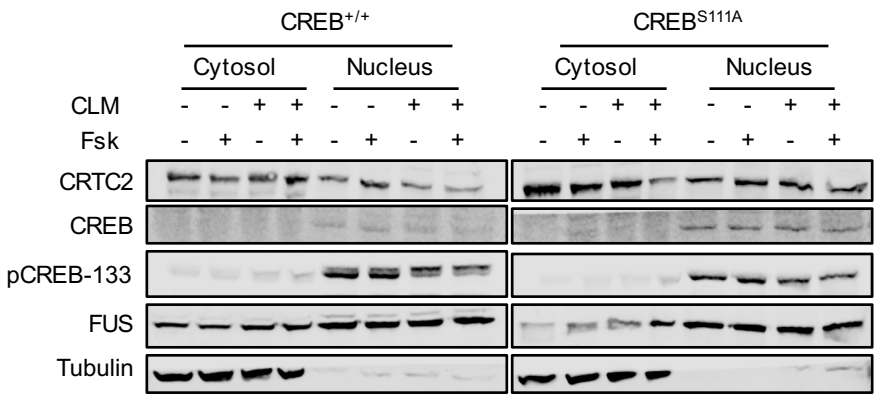


B

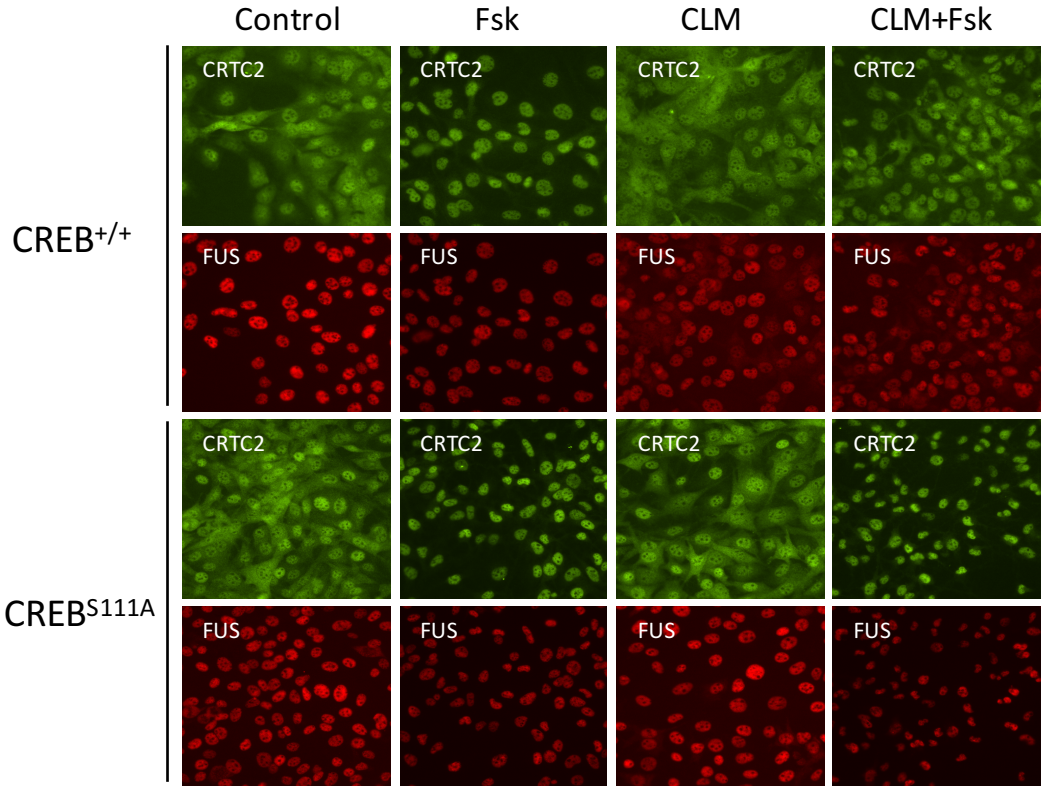
Gene Symbol	CREB WT Fold Change Fsk vs Untreated	CREB S111A Fold Change Fsk vs Untreated
Il6	129.399	95.7367
Cxcl1	100.005	59.5309
Nr4a2	75.3046	27.6409
C2cd4b	70.321	41.3176
Nr4a2	65.9165	30.0651
Areg	35.7718	17.4004
Has1	33.3331	22.6031
chr17:8718223-8949898_F	31.7808	29.9811
Nefl	31.2453	19.1747
Fos	30.2969	17.9173
Rasd1	30.0224	35.6402
Scrt1	29.0702	33.4681
Junb	28.6352	7.5979
Rgs2	25.2571	21.4995
Kcne4	24.9028	13.0753
Apold1	24.6611	17.0207
Ier3	21.6771	7.59684
Nr4a3	19.5444	19.9765
Gem	19.254	11.8194
Gem	17.9409	7.48006
C2cd4a	17.7021	8.61539
Rgs2	17.5774	23.2303
Kcne4	16.7445	11.7443
Ptgs2	16.1898	5.57756
Sik1	15.6338	16.6471

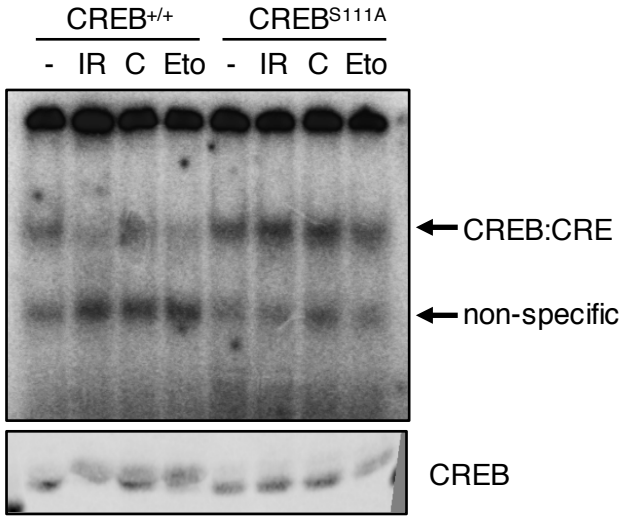

A
CREB
promoter occupancy
0.004
0.0035
0.003
0.0025
0.002
0.0015
0.001
0.0005
0
CREB +/+
CREB S111A
pCREB-133
Promoter Occupancy
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Control
Fsk
20Gy
20Gy-Fsk
CREB +/+
CREB S111A
CBP
promoter occupancy
0.004
0.0035
0.003
0.0025
0.002
0.0015
0.001
0.0005
0
CREB +/+
CREB S111A
CRTC2
promoter occupancy
0.012
0.01
0.008
0.006
0.004
0.002
0
CREB +/+
CREB S111A
B
CREB
Ratio of CREM Promoter Occupancy
5
4
3
2
1
0
CREB +/+
CREB S111A
CRTC2
Ratioof CREM Promoter Occupancy
10
8
6
4
2
0
CREB +/+
CREB S111A
Control
Fsk
CLM
CLM+Fsk

A


CREB+/+
CREBS111A
Cytosol
Nucleus
Cytosol
Nucleus
CLM
Fsk
CRTC2
CREB
pCREB-133
FUS
Tubulin

B


Control
Fsk
CLM
CLM+Fsk
CREB+/+
CREBS111A
CRTC2
FUS


CREB+/+
CREBS111A
- IR C Eto - IR C Eto
CREB:CRE
non-specific
CREB

Supplementary Figure Legends

Supplementary Figure S1.

cAMP-induced phosphorylation of the ATM/CK cluster is independent of S133 phosphorylation. *CREB*$^{+/+}$ and *CREB*^{S133A} MEFs were treated with Fsk, and the cells were collected at the indicated times. Cell extracts were analyzed using α-CREB, or α-pCREB-133 antibodies.

Supplementary Figure S2.

CREB gene targeting strategy

Supplementary Figure S3.

Analysis of CREB target gene expression in *CREB*$^{+/+}$ and *CREB*^{S111A} MEFs. *CREB*$^{+/+}$ and *CREB*^{S111A} MEFs were treated with Fsk for 90 min, irradiated with 20 Gy IR for 120 min (20 Gy), or treated with Fsk for 90 min after 20 Gy IR for 30 min (20 Gy-Fsk). mRNA amounts for HAS2, NR4A1, NR4A3, or GEM in MEFs were analyzed by quantitative real-time PCR. Each bar represents averaged results, n=3. Error bars indicate SEM.

Supplementary Figure S4.

Reduced cAMP-inducible gene expression in *CREB*^{S111A} mice. **(A)** *CREB*$^{+/+}$ and *CREB*^{S111A} MEFs were treated with Fsk for 90 min. mRNA amounts for AREG in MEFs were analyzed by quantitative real-time PCR. Each bar represents averaged results, n=24. Error bars indicate SEM. **(B)** Comparison of the most highly upregulated genes between Fsk-treated *CREB*$^{+/+}$ MEFs and *CREB*^{S111A} MEFs (n=6).

Supplementary Figure S5.

AREG and CREM promoter occupancy between *CREB*$^{+/+}$ and *CREB*^{S111A} MEFs. **(A)** IR-dependent reduction of coactivator recruitment to CREB target gene promoters in primary MEFs. *CREB*$^{+/+}$ and *CREB*^{S111A} MEFs were treated with Fsk for 90 min, irradiated with 20 Gy IR for 120 min (20 Gy), or treated with Fsk for 90 min after 20 Gy IR for 30 min (20 Gy-Fsk). ChIP assays show that occupancy of CREB, pCREB-133, CRTC2 or CBP on the CREB binding site at AREG promoter. Each bar represents averaged results, n=3. Error bars indicate SEM. **(B)** CLM-dependent reduction of coactivator recruitment to the CREB binding site in the CREM promoter. Immortalized *CREB*$^{+/+}$ and *CREB*^{S111A} MEFs were treated with Fsk for 90 min, treated with 2 nM CLM for 120 min (CLM), or treated with Fsk for 90 min after 2 nM CLM for 30 min (CLM+Fsk). ChIP assays show that occupancy of CREB and CRTC2 on the CREB binding site at CREM promoter. Each bar represents averaged results, n=3. Error bars indicate SEM.

Supplementary Figure S6.

cAMP-induced CRTC2 nuclear translocation is resistant to DNA damage. **(A)** Immortalized *CREB*$^{+/+}$ and *CREB*^{S111A} MEFs were treated with Fsk for 90 min, treated with 2 nM CLM for 120 min (CLM), or treated with Fsk for 90 min after 2 nM CLM for 30 min (CLM+Fsk). Cells were fractionated into cytoplasmic or nuclear fractions. The fractionated proteins were analyzed by Western blotting with α-CRTC2, α-CREB, α-pCREB-133, α-FUS or α-β-tubulin antibodies. **(B)** Immortalized *CREB*$^{+/+}$ and *CREB*^{S111A} MEFs were treated with the same condition of (**A**), and the cells were immunostained with α-CRTC2 and α-FUS antibodies.

Supplementary Figure S7.

Mechanistically distinct DNA damaging agents reduced CREB binding to CRE. EMSA was performed using cell lysates from primary *CREB*$^{+/+}$ and *CREB*^{S111A} MEFs treated with IR (20 Gy, 1h), CPT (10 μM, 2 h), or Etoposide (5 μM, 2 h).

Supplementary Table S1A.

Top 50 Fsk-induced genes in *CREB*$^{+/+}$ MEFs

Supplementary Table S1B.

Top 50 Fsk-induced genes in *CREB*^{S111A} MEFs