SUPPLEMENTAL FIGURE LEGENDS

Supplementary Figure 1. Validation of deletion-specific defects of AKAP12. (A) Equal expression of AKAP12-WT and the panel of deletion-mutants (AKAP12ΔPKA, AKAP12ΔATR and AKAP12ΔNLS) in HEK293 cells. (B) AKAP12 in HEK293 cells was silenced by CRISPR-Cas9 genome editing. (C) Immunoprecipitation of HA-tagged AKAP12 wild-type and the panel of mutants (AKAP12ΔPKA, AKAP12ΔATR and AKAP12ΔNLS) and immunoblotting with anti-RII subunit of PKA in HEK293 cells. (D) HEK293 cells expressing either HA-tagged AKAP12 wild-type or the AKAP12ΔPKA mutant were transfected with a plasmid expressing a V5-tagged RII subunit and immunoprecipitated with anti-HA and immunoblotted with anti-V5. (E) Whole cell lysates from HEK293 cells were immunoprecipitated with anti-AKAP12 which was washed in a high salt buffer (0.5M NaCl) to remove any co-precipitated endogenous RII subunit. After collection by centrifugation the bound protein was incubated with recombinant RII (100 ng) for 1 h. The presence of RII binding was confirmed by immunoblotting of anti-RII. (F) Proximity ligation confirming sub-cellular localization and interaction between AKAP12 and the RII subunit of PKA in unirradiated HEK293 cells. Green detection events signify juxtaposition between AKAP12 and the R subunit of PKA. Nuclei were stained with DAPI (blue). (G) Co-IP’s between ATR and HA-tagged AKAP12 wild-type or mutants (AKAP12ΔPKA, AKAP12ΔATR and AKAP12ΔNLS), 30 min post-UV exposure (10 J/m²). (H) Immunoblots of cytoplasmic and nuclear fractions of HEK293 cells expressing wild-type AKAP12 or AKAP12ΔNLS, 30 min post- UV exposure (10 J/m²). (I) Quantitative real-time PCR analysis was performed using an Applied Biosystems 7500 Real Time PCR System (10 ng cDNA/reaction) utilizing TATA-binding protein (TBP) as a reference gene. The relative mRNA abundance of AKAP12 was determined in cells pre-treated with vehicle or forskolin for 30 min and exposed to UVB (10 J/m²). (J) Representative immunoblot showing purity of cytoplasmic, nuclear and chromatin fractions used in experiments.

Supplementary Figure 2. Effect of ATR’s kinase activity on AKAP12 phosphorylation and subcellular localization. (A) Representative immunoblot showing purity of cytoplasmic, nuclear and chromatin
fractions used in experiments. (B, C) HEK293 cells were co-transfected with FLAG-tagged ATR and HA-tagged AKAP12 and administered VE-821 or vehicle. Coimmunoprecipitation was performed using anti-HA with cytoplasmic and nuclear fractions at indicated times after UVB exposure (10 J/m²). An anti-ATR/ATM substrate antibody (an antibody that detects proteins phosphorylated at an ATR/ATM consensus sequences; pSQ/TQ) was used to immunoblot. Input immunoblots represent 10% of whole cellular lysate.

**Supplementary Figure 3.** Enzymatic calculations of ATR-pS435 in vitro kinase assay and antagonism of MC1R signaling by ASIP and HBD3. (A) The $K_m$ and $V_{max}$ kinetic parameters of the ATR-pS435 phosphorylation reaction was calculated by nonlinear regression analysis, using the CPKRRRLSSSLNPS peptide as a substrate. (B, C) HEK293 cells transfected with wild type MC1R were treated with $\alpha$-MSH or forskolin and either agouti signaling protein (ASIP; 100 nM) or human beta-defensin 3 (HBD3; 100 nM) for 30 minutes before quantification of ATR-pS435 levels in whole cell lysates. ATR-pS435 was measured using the peptide, CPKRRRLSSSLNPS as a substrate and an anti-ATR-pS435 antibody coupled with fluorescence detection.

**Supplementary Figure 4.** ATR-phosphorylation of AKAP12 is independent of PKA-mediated phosphorylation of ATR-S435. (A-D) Whole cell lysates were extracted 30 min ($\pm$ 10 J/m² UV, $\pm$ 10 µM forskolin) from ATR-hypomorphic Seckel lymphocytes expressing either wild-type ATR or ATR-S435A. Lysates were immunoprecipitated with anti-AKAP12 and immunoblotted with either an ATR/ATM phosphorylation-specific antibody that detects phosphorylated SQ/TQ motifs or with an ATR-pS435 phosphospecific antibody. ATR kinase activity was inhibited by administration of VE-821 (10 nM). Input of ATR and AKAP12 represents 10% of total lysate.

**Supplementary Figure 5.** PKC does not impact ATR-pS435 generation. (A, B) Confirmation of successful inhibition of PKA and PKC by H-89 (PKA inhibitor) and BIM I (PKC inhibitor). (C-E) Kinase assays using
whole cell lysates were carried out with indicated concentrations of peptide containing S435 and surrounding residues (CPKRRRLSSSLNPS) as a substrate. A375 cells were treated with either forskolin, H-89 (PKA inhibitor) or BIM I (PKC inhibitor) and phosphorylation was measured by immunoslot blot with either, anti-PKA substrate, anti-PKC substrate or anti-pS435 antibodies. The kinetic parameters of the phosphorylation reaction were calculated with nonlinear regression analysis.

**Supplementary Figure 6.** AKAP12 enhances XPA localization to UV-induced DNA damage. (A) Representative immunoblot showing purity of chromatin fractions used in experiments. (B) XPA-[6-4]-PP interactions determined by proximity ligation using anti-XPA and anti-[6-4]-PP antibodies. A375 cells were administered with siRNA-AKAP12 or scrambled siRNA and treated with either vehicle or forskolin (10 µM) and UV exposure. Nuclei and actin stained with DAPI (blue) and phalloidin (pink), respectively. (C-E) Chromatin-associated XPA levels in ATR-hypomorphic Seckel cells transfected with either ATR-WT, ATR-S435A or ATR-S435D and treated with forskolin (10 µM) and exposed to UV. Data are expressed as the percent of chromatin-bound XPA to total cellular XPA. (F) Chromatin-associated XPA levels in A375 cells administered siRNA targeted to either AKAP12, ATR or PKA Cα 30 min after UV. (G) Confirmation of successful siRNA-knockdown of endogenous ATR and expression of Flag-tagged wild-type ATR, ATR-S435A and ATR-S435D. (H, I) AKAP12-CRISPR silenced HEK293 cells expressing either AKAP12-WT or AKAP12ΔPKA were irradiated and chromatin fractions were incubated with an irradiated biotinylated oligonucleotide duplex DNA fragment. Levels of XPA bound to the DNA fragment were quantified by ORiP as described in Materials and Methods. (J) AKAP12-CRISPR silenced HEK293 expressing either AKAP12-WT or AKAP12ΔPKA cells were mock irradiated and chromatin fractions were incubated with an irradiated biotinylated oligonucleotide duplex DNA fragment. Levels of XPA and XPC bound to the DNA fragment were quantified by ORiP as described in Materials and Methods.

**Supplementary Figure 7.** AKAP12 interacts with UV-induced DNA damage. (A) Confocal imaging of AKAP12, XPA and ATR-pS435 in AKAP12-null HEK293 cells treated with either vehicle or forskolin (10
µM) and UV exposure. Cells were exposed to 10 J/m$^2$ of UVC followed by in situ detergent extraction. Co-localization of AKAP12 (pink), XPA (green) and ATR-pS435 (red) is observed as white nuclear foci. (B) Chromatin-associated ATR-pS435 levels in AKAP12-null HEK293 cells transfected with AKAP12-WT, AKAP12-S338A, AKAP12-S505A, AKAP12-S732A or AKAP12-S887A and treated with either Forskolin (10 µM) or vehicle and mock treated or exposed to UV. (C) Localization of potential ATR phosphorylation sites within AKAP12; ATR-interacting, PKA-binding and nuclear localization domains highlighted. Note that S732 lies within AKAP12’s predicted nuclear localization domain.

**Supplementary Figure 8.** XPA, ERCC1-XPF does not bind the ORiP substrate in the absence of UV. (A) AKAP12 CRISPR-deleted HEK293 cells were transfected with wild-type AKAP12 or AKAP12$^{ΔPKA}$, AKAP12$^{ΔATR}$, AKAP12$^{ΔNLS}$ and AKAP12-S732A were treated with either vehicle or forskolin (10 µM). After mock treatment (30 minutes), nuclear lysates were incubated with biotinylated stem-loop substrate for indicated times. Levels of XPA, ERCC1 and XPF bound to the DNA fragment were quantified by ORiP as described in Materials and Methods.

**Supplementary Figure 9.** Forskolin enhances ERCC1 protein levels in UV-exposed chromatin fractions. AKAP12-CRISPR silenced HEK293 cells were either transfected with either (A) AKAP12-WT or (B) AKAP12$^{ΔPKA}$, (C) AKAP12$^{ΔNLS}$, (D) AKAP12$^{ΔATR}$, or (E) AKAP12-S732A. Cells were either mock-treated or exposed to UVB (10 J/m$^2$) and either treated with forskolin (10 µM) or vehicle. After 30 minutes, chromatin fractions were isolated and immunoblots of ERCC1 were performed. Input represents 10% of total cellular lysate.

**Supplementary Figure 10.** ERCC1-XPF is responsible for stem-loop cleavage. (A) Validation of the siRNA-knockdown of ERCC1 or XPG in HEK293 cells. (B) HEK293 cells were either administered scrambled siRNA or siRNA-targeted to ERCC1 or XPG. Cells were exposed to UVB and after 30 minutes, chromatin fractions were incubated with FAM-labeled stem-loop substrate and products visualized on 4-20% polyacrylamide gels.
**Supplementary Figure 11.** Forskolin does not enhance protein levels of XPC, XPB, XPD or XPG in UV-exposed chromatin fractions. AKAP12 CRISPR-deleted HEK293 cells were transfected with wild-type AKAP12 or empty vector and treated with either vehicle or forskolin (10 µM) 30 min before UVB exposure (10 J/m²). After 30 minutes, chromatin fractions were isolated and immunoblots of indicated proteins were performed. Data in all panels are representative from 3 independent experiments and error bars are standard deviation.

**Supplementary Figure 12.** Forskolin enhances XPF incision activity and not XPG incision activity in a bubble substrate. (A) schematic diagram of bubble substrate and incision sites (B) HEK293 cells were either administered scrambled siRNA or siRNA-targeted to XPF or XPG or scrambled siRNA. Cells were exposed to UVB (10 J/m²) and after 30 minutes, chromatin fractions were incubated with FAM-labeled bubble substrate and products visualized on 4-20% polyacrylamide gels. Data in all panels are representative from 2 independent experiments and percent incision ± standard deviation shown.

**Supplementary Figure 13.** MC1R antagonists modulate ERCC1-XPF-mediated DNA incision. MC1R-wild-type expressing cells (SK-MEL2) were pre-treated with forskolin (10 µM), α-MSH (100 nM), α-MSH (100 nM)+ASIP (100 nM) or HBD3 (100 nM) 30 minutes before UV. After 30 minutes, chromatin fractions were incubated with FAM-labeled stem-loop substrate at 37°C for 10 minutes, and products visualized on 4-20% polyacrylamide gels. Treatments significantly different were determined by one-way ANOVA; *p ≤ 0.05. Data in all panels are representative from 3 independent experiments and error bars are standard deviation.
AKAP12 mRNA Expression (Fold Change)

Vehicle
Forskolin
UV
Forskolin + UV

No Treatment
2 hr
4 hr
8 hr
2 hr
4 hr
8 hr
2 hr
4 hr
8 hr

Jarrett et al, Supplementary Figure 1
**A**

| Protein      | Time after UV (min) | Cytoplasm | Nucleus | Chromatin |
|--------------|---------------------|-----------|---------|-----------|
| I.B Tubulin  | 0 5 10 20 30        | 50        |         |           |
| I.B Aly      | 0 5 10 20 30        | 25        |         |           |
| I.B H2B      | 0 5 10 20 30        | 10        |         |           |

**B**

| Protein      | Time after UV (min) | Cytoplasm | Nucleus |
|--------------|---------------------|-----------|---------|
| I.P AKAP12/  | 0 5 10 20 30        |           |         |
| I.B pSQ/TQ   |                     |           |         |
| FLAG-ATR     | 0 5 10 20 30        |           |         |
| HA-AKAP12    | 0 5 10 20 30        |           |         |

**C**

+ VE-821

| Protein      | Time after UV (min) | Cytoplasm | Nucleus |
|--------------|---------------------|-----------|---------|
| I.P AKAP12/  | 0 5 10 20 30        |           |         |
| I.B pSQ/TQ   |                     |           |         |
| FLAG-ATR     | 0 5 10 20 30        |           |         |
| HA-AKAP12    | 0 5 10 20 30        |           |         |
### A

| Cell line               | Vehicle | α-MSH | Forskolin |
|-------------------------|---------|-------|-----------|
|                        | $K_m$   | $V_{max}$ | $K_m$ | $V_{max}$ | $K_m$ | $V_{max}$ |
| AKAP12-WT              |         |       |           |           |       |           |
| MC1R-WT                | n.d.    | n.d.  | 21 ± 3    | 80 ± 12   | 14 ± 3 | 60 ± 13   |
| MC1R-R151C             | n.d.    | n.d.  | n.d       | n.d       | 15 ± 4 | 56 ± 14   |
| MC1R-R294H             | n.d.    | n.d.  | n.d       | n.d       | 13 ± 4 | 56 ± 18   |
| AKAP12$^{\text{PKA}}$ |         |       |           |           |       |           |
| MC1R-WT                | n.d.    | n.d.  | n.d       | n.d       | n.d   | n.d       |
| MC1R-R151C             | n.d.    | n.d.  | n.d       | n.d       | n.d   | n.d       |
| MC1R-R294H             | n.d.    | n.d.  | n.d       | n.d       | n.d   | n.d       |

### B

**AKAP12-WT, MC1R-WT**

- **Forskolin**
- **Forskolin + HBD3**
- **Forskolin + ASIP**

### C

**AKAP12-WT, MC1R-WT**

- **MSH**
- **MSH + HBD3**
- **MSH + ASIP**

*Jarrett et al, Supplementary Figure 3*
### ATR-WT

| Forskolin | -UV | +UV | kDa |
|-----------|-----|-----|-----|
| I.P AKAP12/ I.B pSQ/TQ | ![Image](image1.png) | ![Image](image2.png) | 250 |
| I.P AKAP12/ I.B ATR-pS435 | ![Image](image3.png) | ![Image](image4.png) | 250 |
| Input | ![Image](image5.png) | ![Image](image6.png) | 250 |
| AKAP12 | ![Image](image7.png) | ![Image](image8.png) | 250 |

### ATR-WT + VE-821

| Forskolin | -UV | +UV | kDa |
|-----------|-----|-----|-----|
| I.P AKAP12/ I.B pSQ/TQ | ![Image](image9.png) | ![Image](image10.png) | 250 |
| I.P AKAP12/ I.B ATR-pS435 | ![Image](image11.png) | ![Image](image12.png) | 250 |
| Input | ![Image](image13.png) | ![Image](image14.png) | 250 |
| AKAP12 | ![Image](image15.png) | ![Image](image16.png) | 250 |

### ATR-S435A

| Forskolin | -UV | +UV | kDa |
|-----------|-----|-----|-----|
| I.P AKAP12/ I.B pSQ/TQ | ![Image](image17.png) | ![Image](image18.png) | 250 |
| I.P AKAP12/ I.B ATR-pS435 | ![Image](image19.png) | ![Image](image20.png) | 250 |
| Input | ![Image](image21.png) | ![Image](image22.png) | 250 |
| AKAP12 | ![Image](image23.png) | ![Image](image24.png) | 250 |

### ATR-S435A + VE-821

| Forskolin | -UV | +UV | kDa |
|-----------|-----|-----|-----|
| I.P AKAP12/ I.B pSQ/TQ | ![Image](image25.png) | ![Image](image26.png) | 250 |
| I.P AKAP12/ I.B ATR-pS435 | ![Image](image27.png) | ![Image](image28.png) | 250 |
| Input | ![Image](image29.png) | ![Image](image30.png) | 250 |
| AKAP12 | ![Image](image31.png) | ![Image](image32.png) | 250 |

Jarrett et al, Supplementary Figure 4
Supplementary Figure 5

A. Western blots showing the effects of PMA and BIM I on PKC substrate phosphorylation. I.P p21/1.B PKC Substrate and I.B p21 were analyzed.

B. Western blots showing the effects of forskolin and H-89 on PKC and PKA substrates. I.P CREB/I.B PKA Substrate and I.B CREB were analyzed.

C. Graph showing the PKA phosphorylation of forskolin and forskolin + H-89.

D. Graph showing the PKC phosphorylation of PMA and PMA + BIM I.

E. Graph showing the S435 phosphorylation of forskolin and BIM I. H-89.
A

AKAP12-null

- Forskolin
- UV
+ Forskolin
+ UV

B

| Forskolin | UV | I.B ATR-pS435 | I.B H2A |
|-----------|----|---------------|--------|
| -         | -  |               |        |
| +         | -  |               |        |
| -         | +  |               |        |
| +         | +  |               |        |

kDa
250
10

C

AKAP12

NH$_2$

(1,782 amino acids)

COOH

S388 S505 S732 S887

ATR interaction

Nuclear localization

PKA-R binding

Jarrett et al, Supplementary Figure 7
Jarrett et al, Supplementary Figure 8
A

| Condition | WT | ΔPKA | ΔNLS | ΔATR |
|-----------|----|------|------|------|
| UV        | -  | -    | -    | -    |
| Forskolin | -  | +    | -    | -    |
| ERCC1     | +  | +    | +    | +    |
| H2A       | +  | -    | -    | -    |

B

| Condition | ΔPKA | ΔNLS | ΔATR |
|-----------|------|------|------|
| UV        | -    | -    | -    |
| Forskolin | +    | -    | -    |
| ERCC1     | +    | +    | +    |
| H2A       | +    | +    | +    |

C

| Condition | ΔNLS | ΔATR |
|-----------|------|------|
| UV        | -    | -    |
| Forskolin | +    | -    |
| ERCC1     | +    | +    |
| H2A       | +    | +    |

D

| Condition | ΔATR |
|-----------|------|
| UV        | -    |
| Forskolin | +    |
| ERCC1     | +    |
| H2A       | +    |

E

| Condition | S732A |
|-----------|-------|
| UV        | -     |
| Forskolin | +     |
| ERCC1     | +     |
| H2A       | +     |

Jarrett et al, Supplementary Figure 9
A

| siRNA | - | + | kDa |
|-------|---|---|-----|
| ERCC1 |   |   | 37  |
| Tubulin |   |   | 50  |

| siRNA | - | + | kDa |
|-------|---|---|-----|
| XPG   |   |   | 100 |
| Tubulin |   |   | 50  |

B

| siRNA | - | + |
|-------|---|---|
| ERCC1 |   |   |
| XPG   |   |   |

% Incision

- 47±2.7
- 0.5±0.6
- 48±3.4
- 49±3.7
Jarrett et al, Supplementary Figure 11
Jarrett et al, Supplementary Figure 12
