Supplementary Figure Legends

Figure S1. Effect of EGFR inhibition on short-term survival/proliferation in a NSCLC cell panel. A, illustration of syto60-based screening platform and experimental design. Briefly, ~5,000-10,000 cells were seeded into 24-well plates in complete medium. After overnight incubation, cells were exposed to clinically relevant doses of erlotinib (E) at 2 μM or cetuximab (C) at 100 nM followed by irradiation with 2 Gy 1 hour later. After 72 hours, cells were fixed in 3.8% formaldehyde and stained in a 1:8,000 solution of the fluorescent nucleic acid stain Syto60 (Invitrogen). Quantification of fluorescence was carried out at excitation and emission wavelengths of 630 and 695 nM respectively by using an Odyssey® plate reader. For each data point, cells were seeded in triplicate. SRF_{2Gy}, Short-term Radiosensitization Factor for 2 Gy; NFI, normalized fluorescence intensity relative to control. B, range of SRF2Gy values across a NSCLC cell line panel resistant to erlotinib alone. Solid circles indicate putative radiosensitization. Mean ± SE are based on three biological repeats. KRAS-mutation (mut) status of cell lines is indicated: +, mutant; -, wild-type; ND, not determined. C, correlation of $SRF_{2\ Gy}$ by erlotinib and effect of erlotinib alone on short-term cell survival/proliferation assessed with the Syto60 assay in 40 NSCLC cell lines. D, whole cell lysates of three KRAS-mutant NSCLC cell lines and various NCI-H1703 clones stably transfected with expression vectors for wild-type KRAS, mutant KRAS (G12V), or empty vector control were probed with a specific anti-K-Ras antibody (sc-30, Santa Cruz). *, clones used for further analysis. E, SRF_{2Gy} values were calculated as a function of pre-irradiation drug incubation time (1 hour vs 24 hours) in KRAS mutant (mut) and wild-type (wt) DLD-1 and DWT7 cell lines, respectively. F. Left, representative images showing the effect of IR and erlotinib on NCI-H1703 spheres. Measurement of sphere diameters illustrates drug effect in irradiated spheres. Right, calculation of SRF_{2Gy} values for NCI-H1703 spheres.

Figure S2. EGFR nuclear translocation and NHEJ do not correlate with EGFR-dependent suppression of IR-induced DSBs. A, Left, representative immunofluorescence microscopy images illustrating IR-induced γ-H2AX foci with or with cetuximab treatment (100 nM), corresponding to Fig. 2A. Right, percentage of A549 cells with ≥ 20 γH2AX foci/nucleus over time post-irradiation with or without pretreatment of erlotinib at 2 μM. B, representative immunofluorescence microscopy images of nuclear translocation of EGFR induced by irradiation of A549 cells, which was not affected by cetuximab. Cells were irradiated with 8 Gy +/- pre-incubation with cetuximab (100 nM). Cells were fixed with 4% PFA without permeabilization and incubated with anti-EGFR antibody (EGFR (1005), 1:200 dilution, Santa Cruz) for 2 hours at room temperature, and subsequently incubated with an Alexa488 secondary antibody (Invitrogen) for 1 hour at room temperature. Images are overlays of DAPI and anti-EGFR stain (green). C, EGFR activity did not affect NHEJ frequencies in a GFP-based I-SceI reporter assay (Mansour MY, et al. (2008) Hierarchy of non-homologous end-joining, single-strand annealing and gene conversion at site-directed DNA double-strand breaks. Nucleic Acids Res 36:4088-4098). Irradiated A549 cells were co-transfected with a NHEJ reporter and an expression vector for I-SceI endonuclease, with or without treatment with 100 nM cetuximab. Forty-eight hours later, the percentage of green fluorescent recombinations was determined relative to a control for transfection efficiency. Bars represent the mean of three biological repeats.

Figure S3. EGFR and PKCα are co-regulators of Aurora B kinase-mediated chromatin condensation in interphase. A, no promoting effect of EGFR on methyltransferase activity in A549 cells. The activity of H3K9 methyltransferase in A549 cells after EGF stimulation +/- treatment with erlotinib was detected by using the EpiQuik™ Histone Methyltransferase Activity/Inhibition Assay Kit (H3K9) (Epigentek, Brooklyn, P-3003) which uses recombinant G9A as the control enzyme. The assay was performed following to the manufacturer's protocol. Bars represent mean +/- standard error based on three biological repeats. B, representative immunofluorescence microscopy images from A549 xenografts +/- erlotinib treatment, stained with the dual H3K9me3/phospho-H3S10 antibody. C, representative immunofluorescence images showing co-localized phospho-H3S10 and H3K9me3 by using a specific dual antibody in A549 cells +/- treatment with erlotinib or the Aurora B kinase inhibitor hesperadin. D, percentage of A549 cells with ≥ 20 γH2AX foci/nucleus 30 minutes after 1 Gy irradiation with a panel of pharmacological inhibitors: PKC412 (Sigma-Aldrich) 100 nM; AZD6244 (Selleck) 250 nM; RAD-001 (LC Laboratories) 20 nM; BEZ235 (Selleck) 250 nM; P6 (Selleck) 5 μM. E, representative immunofluorescence images showing co-localized phospho-H3S10 and H3K9me3 by using a specific dual antibody in A549 cells +/- erlotinib or +/- the specific PKCα̃/β inhibitor Gö6976. F, percentage of NCI-H1703 cells with or without mutant K-Ras expression showing ≥ 20 γH2AX foci/nucleus 30 minutes after 1 Gy irradiation with or without pharmacological PKCα inhibition.

Figure S4. A, KRAS wild-type cells are sensitive to erlotinib alone. Effect of erlotinib alone on short-term cell survival/proliferation assessed with the syto60 assay in DLD1 (KRAS wt/mut) or DWT7 (wt/-) CRC cells. B, combined inhibition of both MEK-ERK and PKCα produced the full senescence phenotype induced by EGFR inhibition after radiation. Representative images (40X) showing staining for DAPI and senescence-associated β-galactosidase (SA-β-gal) 7 days following 2 Gy irradiation in A549 cells +/- erlotinib, AZD6244, or Gö6976. C, Left, representative FACS histograms illustrating the sub-G1 fraction in A549 cells 72 hours following irradiation. Right, percentage of sub-G1 cells for the treatments indicated. D, SRF_{2Gy} values for A549 cells treated with drugs as indicated. Statistical comparison with unpaired T-test. E, representative images of irradiated DLD-1 (KRAS wt/mut) and DWT7 (wt/-) spheres complementing Fig. 5F. Non-irradiated spheres not shown. Arrows indicate combined effect of chloroquine (CQ) and erlotinib which is a result of underlying apoptosis induction.