Figure Legends for Supplemental Section

Supplemental Figure 1: DNA synthesis, mitosis and apoptosis in wild-type EGFR NSCLCs in response to radiation. (A) BrDU positive nuclei were counted at 18 hours following IR at indicated doses in 4 wild-type EGFR expressing cell lines by immunocytochemistry with anti-BrDU primary and Cy-5 labeled secondary antibody. Mean values and standard deviations were calculated from 15 different fields, in triplicate wells, representing > 8000 nuclear events (B) Mitotic fractions were identified in 5 wild-type NSCLC cell lines in response to indicated doses of IR by immunocytochemistry with anti-phospho histone H3 antibody and Cy5-labeled secondary antibody. Mean and standard deviation values (error bars) in the figure are representative of > 5000 nuclear events from 24 fields in duplicate wells. (C) Apoptotic nuclei were detected in 5 wild-type EGFR expressing cell lines by the presence of high intensity apoptotic bodies. Percent apoptotic nuclei were plotted as a function of radiation dose. Mean values (solid bars) and standard deviations (error bars) are a representative of > 5000 nuclear events from 60 fields in 6 different wells.

Supplemental Figure 2. *Radiation induces micronuclei formation in mutant EGFR but not wild-type expressing NSCLC cell lines.* Twenty-four hours following IR, medium was replaced and cells were maintained in cytochlasin B containing medium for 28 hours and then fixed and stained with DAPI. Images were acquired at 20x magnification on the In Cell Analyzer 1000 and micronuclei were scored using Developer Toolbox software on the basis of the following criteria (a) 3-7 μm diameter (b) < 10 μm distance from mitotic binuclei (c) intensity of DAPI fluorescence equal that of mitotic nuclei. Micronuclei (arrows) per 100 cells was plotted as a function of radiation dose.

(A) Images of DAPI stained nuclei from 2 representative cell lines, A549 and H820 are shown. (B) Micronuclei index was calculated as number of micronuclei per 100 nuclei and plotted as a function of radiation dose. Mean values (solid bars) and standard deviations (error bars) are a representative of > 3000 nuclear events from 30 fields in 6 different wells.

Supplemental Figure 3. Ectopic expression of mutant EGFR does not sensitize H1299 cells to gefitinib or erlotinib. Cell viability was examined in H1299 NSCLC cells stably expressing a LacZ vector, wild-type EGFR, L858R or ΔE746-E750 mutant EGFR in response to a dose range of (A) gefinib or (B) erlotinib by MTS (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega, Madison, WI) assays according the manufacturer's instructions. Inset shows levels of EGFR expression in various transfectants. Cells were plated 24 hrs prior to addition of gefitinib or erlotinib. Absorbance measurements were determined at 490 nm for MTS 96 hrs after addition of drug. Assays were performed in duplicate 96 well plates until a minimum of three plates produced a standard deviation smaller than the mean Assays were performed in duplicate 96 well plates until a minimum of three plates produced a standard deviation (error bars) smaller than the mean. Insets in A-E reveal assessment of EGFR expression by western blot analysis with anti-EGFR antibody.