SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. Role of KRAS status for radioresistance.
A, Under IRB-approved protocol, we reviewed the data on 37 patients with primary or recurrent NSCLC cancer with known KRAS genotype who underwent definitive radiation therapy as primary or adjuvant treatment at MGH (Mak RH, et al., Int J Radiat Oncol Biol Phys. 2010;78:abstract #76.) Freedom from recurrence rate was calculated from the date of diagnosis to the date relapse or to the date of last follow-up. Overall survival rate was calculated from the date of diagnosis to either the date of death or the date of last follow-up if the patient was still living at the time of analysis. Shown are Kaplan-Meier curves for freedom from recurrence and overall survival. Statistical comparison by logrank test (two-sided). mut, mutated; wt, wild-type.
B, Western blot showing the expression of KRAS, AKT, ERK and the phosphorylation of AKT and ERK in the isogenic KRASmut and KRASwt NCI-H1703 NSCLC model. Total KRAS, AKT, and ERK expression were assessed using anti-KRAS antibody (sc-30;Santa Cruz, Dallas, TX), anti-AKT and anti-ERK antibody (#9272, #9102, Cell Signaling, Danvers, MA), respectively. Phosphorylation of AKT and ERK were assessed using anti-phospho-AKT (Ser473) and anti-phospho-ERK antibody (#4058, #9101, Cell Signaling, Danvers, MA), respectively.
C, Cell-cycle distributions of KRAS mut and wt spheres and monolayer culture were determined using standard ethanol fixation and propidium iodide (Sigma-Aldrich) staining. For each condition, 10^4 cells were analyzed by a LSRII flow cytometer (BD Biosciences, San Jose, CA, USA). Representative FACS histograms are shown. Percentages of cells in G1, S, and G2/M phases are averages based on three repeat experiments.
D, Effects of erlotinib and single-dose (1x 2 Gy) or fractionated (5x 2 Gy) IR on cell survival in NCI-H1703 sphere cultures, providing the data for calculation of radiosensitization factors shown in Figure 1H.

Fig. S2. CSC-like features of KRASmut NSCLC cells.
A, Representative FACS dot plots of CD133 staining. Cells were incubated with monoclonal CD133/1 (CD133)-PE conjugate antibody (1:11) at 4°C in the dark for 30 minutes. The cells were then analyzed by flow cytometry. Autofluorescence controls were run in parallel. An arbitrary gate for "high" CD133 expressors was set based on untreated control cells where the relative percentage of cells with high CD133 intensity yielded 0% in the unstained cells and ~2.5% in stained 2D cultured A549 cells. Corresponding values were ~14% and ~45% in NCI-H1703 spheres with wt and mut KRAS, respectively.
B, Whole cell lysates of KRASmut NCI-H1703 spheres were subjected to Western blotting with antibodies against vimentin (#5741, Cell Signaling) and β-catenin (#8480 Cell Signaling).
C, Illustration of sphere size following seeding of 5,000 NCI-H1703 KRASmut or wt cells sorted for either high or low CD133 expression.
D, Left panel, A549 monolayer population containing ~10% of cells with MLCC were subjected to 8 Gy irradiation and concurrent 2 μM erlotinib treatment to eliminate MLCC expression. Following irradiation, cells were allowed to form colonies in the presence of erlotinib. Single colonies were expanded in media without erlotinib to generate independent clones. Right panel, percentage of cells with MLCC in a representative clonally derived cell line (CII) showing rapid re-acquirement of MLCC by passage 2 at levels that were higher than pre-irradiation.
E, Left panel, representative images of A549 CII cells re-challenged with erlotinib 2 μM for 1 hour at passage 2, illustrating how the MLCC fraction (interphase-like punctate staining pattern) has retained sensitivity to erlotinib. Right panel, quantification of findings.

F, Treatment of KRASmut NCI-H1703 spheres with one 2 Gy fraction or 5 daily fractions of 2 Gy +/- erlotinib 2 μM treatment which started 18h prior to irradiation. CD133 expression was determined 10 days after start of irradiation. Bars represent mean with standard error based on 3 independent repeat experiments. Figure complements Fig. 1H.

Fig. S3. Pathway analysis of differentially expressed genes in KRAS mut vs wt NCI-H1703 cells. See Materials and Methods in the main text.

Fig. S4. Factors influencing the radioresistance of NSCLC cell lines with mut KRAS.
A, Western blot showing successful osteopontin (OPN) knockdown in 6 NSCLC cell lines with differing KRAS status.
B, Fraction of cells transfected with control (Con) or SPP1 siRNA, containing at least 20 53BP1 foci at 24h after 8 Gy IR. Bars represent mean +/- standard error based on four independent repeat experiments; *, $p \leq 0.05$ (T-test).
C, Whole cell lysates of KRASmut NCI-H1703 cells compared to wt controls following treatment of cells with recombinant OPN (1 μg/mL) or 2 μM erlotinib for 1h, subjected to Western blotting with antibodies against H3K9me3, total H3, and β-actin.
D, Fraction of cells, transfected with siRNA or/and treated with a histone methyl-transferase inhibitor (HMTi, Chaetocin at 100nM) as indicated, containing at least 20 53BP1 foci 15 minutes after 1 Gy IR. Bars represent means with standard error from 3 independent repeats.
E, Whole cell lysates of KRASmut NCI-H1703 cells compared to wt controls, treated with PI3K inhibitor LY294002, PI3K/mTOR inhibitor BEZ235, and MEK inhibitor AZD6244 (LC Laboratory, Woburn, MA) with concentrations as indicated for 1h, were subjected to Western blotting with antibodies against phospho-AKT, total AKT, phospho-ERK, total ERK and β-actin as published (Wang M, et al.,Cancer Res. 2011;71:6261-9).
F, Clonogenic survival fractions of NCI-H1703 cells with or without treatment with 10 μM LY294002, 250 nM BEZ235, 250 nM AZD6244, or 10 μM DNA-PKcs inhibitor NU7026 (EMD Millipore, Billerica, MA) for 1h prior to 6 Gy IR. NU7026 was used at a standard concentration known to inhibit DNA-PKcs and cause radiosensitization in a variety of cancer cell lines, including in our previous work (Mukherjee, B., et al., Neoplasia 2012;14(1): 34–43; Wang M., et al., Cancer Res. 2014;74:2825-34; Ma. H., et al. Radiat Oncol. 2015;10:225). Bars represent means +/- standard error based on 3-5 independent repeat experiments.

Fig. S5. Role of SPP1/osteopontin expression in KRASmut and wt NSCLC cell lines.
A, *SPP1* expression (RNA seq) in 230 lung adenocarcinomas and associated normal tissues, if available, obtained from The Cancer Genome Atlas (TCGA). Horizontal lines indicate median. ns, not significant.
B, Effect of siSPP1 or siCon transfection on DSB induction in KRASmut and wt NSCLC cell lines subjected to 1 Gy IR. Simultaneous treatment with erlotinib and chaetocin as in Fig. S4. Fraction of cells with at least 20 γ-H2AX foci is shown for the different treatments as indicated. Bars represent mean with standard error based on 3 independent repeats.
C, Analogously to panel B, impact of siSPP1 and siCon transfection and inhibitor treatments in the same 4 cell lines.
D, Percentage of SW1573 cells expressing MLCC after inhibitor treatments (2 μM erlotinib or 1 μM saracatinib) as indicated.

Fig. S6. Role of MLCC in KRASmut NSCLC.
A, Illustration of *CRMP1* gene cluster complementing Fig. 5A.
B, Oncoprint from cBioPortal depicting alterations in candidate genes hypothesized to promote MLCC expression (i.e., *SPP1*, *SRC*, and *EGFR* reported in the current study, *AURKB* and *PRKCA* reported previously (Wang M, et al., Cancer Res. 2014;74:2825-34), plus *ITGB3* reported to interact with OPN to promote aggressive malignant behavior (Furger et al. Mol Cancer Res. 2003;Sep;1(11):810-9) in a cohort of 75 KRASmut lung adenocarcinomas obtained from the TCGA.

Fig. S7. BIM regulation in KRASmut NSCLC.
A, *BIM* expression in 75 KRASmut lung adenocarcinomas with putative MLCC pathway altered or unaltered (see Fig. S6B). Data taken from TCGA. Data represent mean +/- standard deviation. Statistical comparison by T-test.
B, Fold-change of *BIM* expression in KRASmut A549 cells following treatment with 1 µM erlotinib for 6-48h. Data were obtained from www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE57422.
C, Representative immunofluorescence microscopy images illustrating BIM expression in tumor xenografts from KRASmut versus wt NCI-H1703 cells following ex-vivo treatment with 2 µM erlotinib or/and 1 µM saracatinib for 24h.
D, Cell survival fractions of KRASmut NCI-H1703 tumor spheres compared to wt controls 5 days after treatment with 1, 2.5, or 5 µM ABT-263 for 18h and 2 Gy IR. Survival was assessed using the short-term CellTiterGlo assay. Bars represent means +/- standard error based on three independent repeat experiments. Statistical comparisons were performed with the T-test.
E, Short-term radiosensitization factors (SRF_{2Gy}) values for 1 and 5 µM ABT-263 in KRASmut and wt NCI-H1703 tumor spheres. SRF_{2Gy} factors were derived as described (Liu Q, et al., Mol Cancer Res. 2015;13:713-20).
F, Correlation of curated TP53 hotspot mutations and SPP1 expression in a cohort of KRASmut NSCLCs (shown in Fig. S6B), based on the finding of enrichment for TP53 mutations in the MLCC positive subset of KRASmut tumors shown in Fig. 5. Statistical comparison by Mann-Whitney test, one-sided.