**SUPPLEMENTARY FIGURE LEGENDS**

**Figure S1.** Cisplatin sensitivity as a function of EGFR status. A, Upper panel, Western blot showing human EGFR protein levels in A549 cells with endogenous wild-type EGFR 48 hours after transient transfection with an empty control vector or an expression vector for mutant (mut) EGFR (L858R) (upper panel), analogous to Figure 4D. Lower panel, analogous Western blot for EGFR-mutant PC9 cells transiently transfected with wild-type (wt) EGFR. B, Transient transfection efficiency expressed as percentage of GFP-positive cells following parallel transfection of pEGFP-N1 (Invitrogen). C, Clonogenic survival fraction of A549 cells treated with 8 μM cisplatin where expression of mutant EGFR causes a ~1.8-fold reduction in survival, not accounting for transfection efficiency of ~50%. Data points represent means +/- standard error based on 2 biological repeats. PC9 cells transfected with wild-type EGFR did not form colonies. D, Short-term cell survival using fluorescence staining (with syto60) at 72 hours after cisplatin treatment with 8 μM for 1 hour. Y-axis display fold change in cell survival upon manipulation of EGFR status normalized for transient transfection efficiency, which is about 1.5-fold for either cell line. E, Fraction of cells with residual γ–H2AX foci correlates with clonogenic survival after cisplatin treatment. Shown are representative images, corresponding to Fig. 1D. Twenty-four hours after treatment cells were fixed and stained with anti-γ–H2AX antibody (green) and nuclei visualized with DAPI (blue). Cell lines are ranked according to decreasing clonogenic survival after treatment with 8 μM cisplatin (illustrated by grey triangle on the left-hand side). E, Acquired resistance to EGFR TKI (erlotinib or gefitinib) in PC9 cells with mutant EGFR maintains cisplatin sensitivity relative cell lines with wild-type EGFR (solid lines). Clones IR7 (gefitinib), gtpPC9 (gefitinib), gtPC9 #4 (all gefitinib resistant), etPC9 (erlotinib-resistant) were derived by prolonged exposure to TKI as described by Sharma et al. (1). Cells were treated with cisplatin at the indicated concentration for 1 hour. Data points represent mean +/- standard error based on 2-3 biological repeats. F, EGFR-mutant P9 cells "age" with prolonged passaging (> 20 passages) affecting EGFR TKI sensitivity as well as cisplatin sensitivity

(data not shown). Sensitivity of various PC9 culture to erlotinib was assessed using the Syto60 assay (1). Green curve represents parental PC9 culture with low passage (< 20) used for the current study including panel E, demonstrating an IC50 concentration similar to published value of approximately (0.02 μM). Red lines represent PC9 cultures of varying high passage numbers (> 20) exhibiting increased resistance to erlotinib. For comparison, black curves depict PC9 clones with acquired erlotinib resistance following continuous exposure to 2 μM erlotinib for 2 months according to the published protocol (1).

**Figure S2.** Fanconi Anemia (FA) phenotype of EGFR-mutant lung cancer cell lines. A, Flow cytometry demonstrating crosslinker-induced G2/M arrest, complementing Figure 2B. The G2/M arrest seen in FANCD2-mutant PD20 fibroblasts treated with mitomycin C (MMC) at 25 ng/ml for 24 hours is comparable to the cell cycle profile obtained with the EGFR-mutant cell line PC14. B, Fraction of cells with at least 10 subnuclear phospho-ATM foci. Cells were treated with 8 μM cisplatin for 1 hour and fixed 24 hours after treatment. Cells were stained for pATM using anti-pATM antibody (#200-301-400, Rockland). Bars depict means ± standard error based on two biological repeats. Increased levels of pATM foci in mutant EGFR cell lines PC9 and PC14 correlate with cellular FA phenotype.

**Figure S3.** Intact FANCD2 foci formation in EGFR-mutant cells. A, Representative immunofluorescence microscopy images of intact subnuclear FANCD2 foci formation corresponding to Fig. 3A. B, Western blot using a gradient gel, as published previously (2), demonstrating intact FANCD2 mono-ubiquitination in PC9 cells. Mono-ubiquitination in PC9 cells appears to peak at 24 hours compared to 48 hours in H1703 cells. Whether the decline in mono-ubiquitination in PC9 cells at 48 hours is due to increased cell death was not investigated further. FANCD2-L, long mono-ubiquitinated form; FANCD2-S, short unmodified protein form. C, Representative images of subnuclear BRCA1 foci formation, which is upstream of FANCD2 foci,

with and without treatment with 8 μM cisplatin for 1 hour. BRCA1 staining was performed as described previously (2).

**Figure S4.** Analysis of FANCD2-dependent ICL repair. A, Upper panel, Determining ICL repair using a modified alkaline Comet assay. Representative images show comets 5 hours after treatment of PC9 cells with cisplatin, corresponding to Figure 3B. In this modified neutral comet assay, adapted from ref. (3), cells were treated with 50 μM cisplatin for 1 hour and/or irradiated with 12.5 Gy ionizing radiation (IR) just before harvesting. Cells were irradiated to introduce a known amount of DNA double-strand breaks. ICLs retain the double-stranded DNA, thereby shortening the tail moment. Complete unhooking would, therefore, result in the same tail moment as for irradiated only controls. The smaller the tail of cisplatin/IR-treated cells, the less unhooking occurred. Tails of mutant EGFR cells treated with cisplatin/IR are shorter than those in wild-type EGFR cells, indicating reduced unhooking of ICLs. To quantify the amount of ICLs remaining unhooked in the cell, we employed the following formula:

$$\text{ICL remaining [\%]} = \left(1 - \frac{\text{Cp.IR} - \varnothing}{\text{IR} - \varnothing}\right) \times 100\ \%$$

Where Cp.IR = tail moment of cisplatin treated and irradiated cells; ∅ = tail moment of untreated cells; and IR = tail moment of irradiated cells. Lower Panel, Quantification of remaining ICLs in % in the isogenic NIH-3T3 cell pair with or without stable expression of mutant EGFR, complementing Fig. 3B. Black arrow indicates time point when treatment with cisplatin was initiated. The largest difference of unhooked ICLs can be seen at 5 hours after starting treatment, with significant delay of unhooking in the mutant line even after 24 hours. Data points represent means ± standard error based on 2 biological repeats. B, Illustration of increased replication fork stalling or collapsing in EGFR mutant PC9 cells. Shown is the fraction of cells with ≥10 replication protein A (RPA) foci 5 hours after treatment with 8 μM cisplatin for 1 hour. Bars represent means ± standard error based on 2-3 biological repeats. C, Gene expression of factors involved in ICL

unhooking. Microarray expression data were obtained from the publicly available Cancer Cell Line Encyclopedia (CCLE) at http://www.broadinstitute.org/ccle/home. The relative gene expression is shown on the y-axis. Cell lines are grouped into EGFR wild-type versus mutant based on the information in the data base. Horizontal lines represent the mean gene expression in each group. P-values are based on a two-tailed T-test. With a p=0.009, FAN1 nuclease expression was the only factor that was significantly lower expressed in the EGFR mutant group compared to wild-type. D, Western blot of FAN1 protein expression in wild-type and mutant EGFR lung cancer cell lines. FAN1 protein was detected in whole cell lysates using anti-FAN1 antibody (#ab95171, Abcam). FAN1 protein is expressed in all cell lines to varying degree, with a trend towards lower expression in the cell lines with known EGFR mutation. E, DNA repair defect caused by EGFR mutation is epistatic with FANCD2. Mutant EGFR increases the number of γ–H2AX foci in FANCD2 wild-type but not mutant cells after treatment with mitomycin C (MMC), corresponding to Fig. 3D. The isogenic PD20 cell pair either mutant (mut) for FANCD2 or complemented with wild-type (wt) FANCD2 was transfected with expression vectors coding for wild-type or mutant EGFP together with GFP at a 5:1 molar ratio. Forty-eight hours after transfection, cells were treated with 1 μg/ml MMC for 1 hour. At 24 hours, cells were fixed, permeabilized, and stained for γ–H2AX using the standard protocol. Only successfully transfected cells expressing GFP were scored.

**Figure S5.** Analysis of crosslinker-induced RAD51 foci formation as a function of EGFR status. A, Crosslinker-induced RAD51 foci formation is impaired in FANCD2 mutant cells, comparable to our previously published findings (2, 4). PD20 cells were treated with 1 μg/ml MMC for 1 hour and analyzed for RAD51 foci at 5 hours. Bars represent means ± standard error based on 3 biological repeats. B, Representative images of defective subnuclear RAD51 foci formation in a subset of EGFR-mutant lung cancer cell lines, overlaid with DAPI staining, in EGFR wild-type and mutant lung cancer cell lines, corresponding to Figure 4A. Cells were treated with 8 μM cisplatin for 1 hour and stained for RAD51 at 5 hours. C, Quantification of fraction of cells with ≥10 RAD51 foci,

complementing Fig. 4A. Bars represent means ± standard error based on 2-3 biological repeats. D, RAD51 protein is expressed similarly across all cell lines analyzed regardless of EGFR mutation status. Whole cell lysates were obtained and RAD51 protein levels were detected using anti-RAD51 antibody (GTX70230, GeneTex) as published previously (2). E, EGFR-mutant PC9 cells are proficient in homology-directed repair of DSB induced by the I-SceI meganuclease. Cells were transfected with the pDR-GFP substrate together with an I-SceI expression vector at a 1:5 molar ratio as described previously (5). Green fluorescent cells were identified by FACS at 48 hours. The HRR frequency was determined by correcting for transfection efficiency based on parallel transfection with pEGFP-N1. Representative flow histograms are shown.

**Figure S6.** EGFR nuclear translocation and DNA-PKcs status do not correlate with cisplatin sensitivity. A, Nuclear translocation of wild-type EGFR was induced by cisplatin in A549 cells but was resistant to EGFR inhibition and did not correlate with the magnitude of the repair defect seen in Fig. 2-4. Cells were treated by cisplatin (8 μM) for 5 hours, with or without erlotinib (2 μM) or cetuximab (100 nM). Following fixing with 4% PFA without permeabilization, cells were 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 and illustrate nuclear translocation of EGFR. Percentages indicate mean +/- standard error based on two biological repeats. B, Pharmacological DNA-PKcs inhibition has no influence on cellular sensitivity to cisplatin. The isogenic NIH-3T3 cell pair with wild-type (wt) or mutant (mut) EGFR was treated with increasing doses of cisplatin for 1 hour and clonogenic survival was determined analogous to Figure 1. For combination treatment with a DNA-PKcs inhibitor (NU7026, #260961, Calbiochem), cells were treated with 10 μM of NU7026 for a total of 25 hours in addition to 1 hour of cisplatin treatment starting after 1 hour pre-incubation of NU7026. Following wash-off of NU7025 at 25

hours, cells were allowed to form colonies for 2 weeks. Data points represent means ± standard error based on 2-3 biological repeats.

**Figure S7.** Kinase-independent regulation of DNA repair by EGFR. A, Inhibition of EGFR downstream signaling with the PI3K inhibitor LY294002 (L-7962, LC Laboratories). In order to assess effective inhibition of EGFR downstream signalling, a wild-type (wt) EGFR cell line (A549) and a mutant (mut) cell line (PC9) were treated with two different doses of the PI3K inhibitor. Phosphorylation of AKT was evaluated using anti-phospho-AKT (Ser473) antibody (#4058, Cell Signaling). Total AKT expression was assessed using anti-AKT antibody (#9272, Cell Signaling). Phosphorylation of AKT decreased markedly in cells treated with 50 μM LY294002 for 2 hours and almost vanished with 100 μM. B, Disrupting PI3K-Akt axis does not rescue RAD51 foci formation, analogous to Fig. 4C. Black bars: Control samples were untreated or treated with 8 μM cisplatin for 1 hour and RAD51 foci were analyzed at 5 hours. Alternatively, cells were pretreated with 50 or 100 μM PI3K inhibitor for either 2 or 19 hours prior to adding cisplatin. Cells were maintained in PI3K inhibitor until fixation at 5 hours after cisplatin treatment. Bars represent means ± standard error based on two biological repeats. C, Inhibition of EGFR phosphorylation and downstream signalling in EGFR-mutant PC9 cells, complementing Fig. 4C. Western blot demonstrates that erlotinib concentrations between 0.01-5 μM decrease phospho-EGFR levels to varying degrees as expected. The effect on p-ERK was more pronounced than on p-Akt. Antibodies used were against: phospho-EGFR (#44-786G, Biosource), total EGFR (sc-03, Santa Cruz), phosphor-Akt, total AKT (see above), phospho-ERK (#9101, Cell Signaling), and total ERK (#9102, Cell Signaling). D, Erlotinib does not sensitize EGFR wild-type A549 cells to cisplatin. Shown is the fraction of cells with at least 20 γ-H2AX foci as surrogate marker of cisplatin sensitivity with or without 2 μM erlotinib treatment and for two different mitomcyin C (MMC) concentrations. Data points represent means ± standard error based on two biological repeats. E, Transfection of A549 cells with mutant EGFR, analogous to Fig. S1A-D, but not kinase inhibition, suppresses RAD51 foci formation in

response to cisplatin treatment. Note that only ~50% of cells are transfected (Fig. S1B), meaning that the true RAD51 foci suppressing effect is two-fold higher than depicted in the Figure. Bars represent mean +/- standard error based on 2 biological repeats. F, Working model illustrating the hypothesis of a competing relationship of mutant and wild-type EGFR. Either dominant-negative mutant EGFR or depletion of wild-type EGFR disrupts FAN1 and RAD51 recruitment leading to defective homologous recombination repair (HRR).

**Figure S8.** Olaparib sensitivity of EGFR mutant cells. A, Short-term survival of A549 cells with or without depleted wild-type EGFR in response to 10 μM olaparib, analogous to Figure 4F. Bars represent mean +/- standard error based on 2 biological repeats. B, Subnuclear γ–H2AX staining correlates with increasing cellular sensitivity to olaparib, complementing Figure 5C and 5D. Cell lines are ranked from top to bottom by increasing sensitivity to olaparib. Representative low-power images with overlaid DAPI and γ–H2AX stain are shown. C, Ex-vivo assay for assessing DNA double-strand break formation in response to olaparib. Fresh tumor tissue is obtained from core biopsies or other methods. Viable tumor tissue not required for pathological diagnosis is placed in RPMI medium and hand-carried to the laboratory where it is aliquoted. Samples are subjected to mock or olaparib (10 μM) treatment for 24 hours. Samples are then snap frozen in OCT using our published protocol and processed (see main text and references therein) for details. D, Representative low-power images of a core biopsy section from a patient with an EGFR-mutant lung adenocarcinoma, complementing Figure 5D. Arrows indicate 2 cells double positive for a subnuclear PCNA staining patterns that is consistent with S-phase and γ-H2AX foci.