Supplementary Figures

FIGURE S1. Representative images to illustrate RAD51 foci induction in FANCD2 wild-type (wt) and mutant (mut) cells. Higher magnification is shown in Figure 1A. Arrows indicate cisplatin-induced foci. Blue, DAPI; red, PCNA; green, RAD51.

FIGURE S2. Cell-cycle distributions were determined using standard ethanol fixation and propidium iodide (Sigma-Aldrich) staining followed by flow cytometry at a core facility at the Ragon Institute at MGH.

FIGURE S3. Correlation of ERCC1 gene expression with cisplatin-induced RAD51 foci. Linear regression line is shown. Gene expression data were obtained from the publicly available Cancer Cell Line Encyclopedia (CCLE). http://www.broadinstitute.org/ccle/home

FIGURE S4. Role of ATM in cisplatin resistance. A, Representative images illustrating reduced ATM phosphorylation in H2087 cells 24 hours after treatment with 8 μM cisplatin. Phospho-ATM was visualized with mouse anti-ATM pS1981 monoclonal antibody (Rockland #200-301-400) at a 1:100 dilution. B. Clonogenic cell survival following cisplatin treatment. Left panel, A549 cells were mock treated or treated with the ATM kinase inhibitor KU55933 at 20 μM (Chemdea). Right panel, mouse embryonic fibroblasts (MEFs) with the indicated genotypes. Data represent means +/- standard error based on 2-3 biological repeats. C, Time course of γ-H2AX and p-ATM (S1981) foci in A549 and Calu-6 cells following treatment with 8 μM cisplatin for 1 hour. D, Co-staining of PCNA and p-ATM foci in A549 cells treated with 8 μM cisplatin. To eliminate non-chromatin bound PCNA, cells were permeabilized first with 0.5% Triton-X, 20mM HEPES, 50mM NaCl, 3mM KCl, 300mM Sucrose on

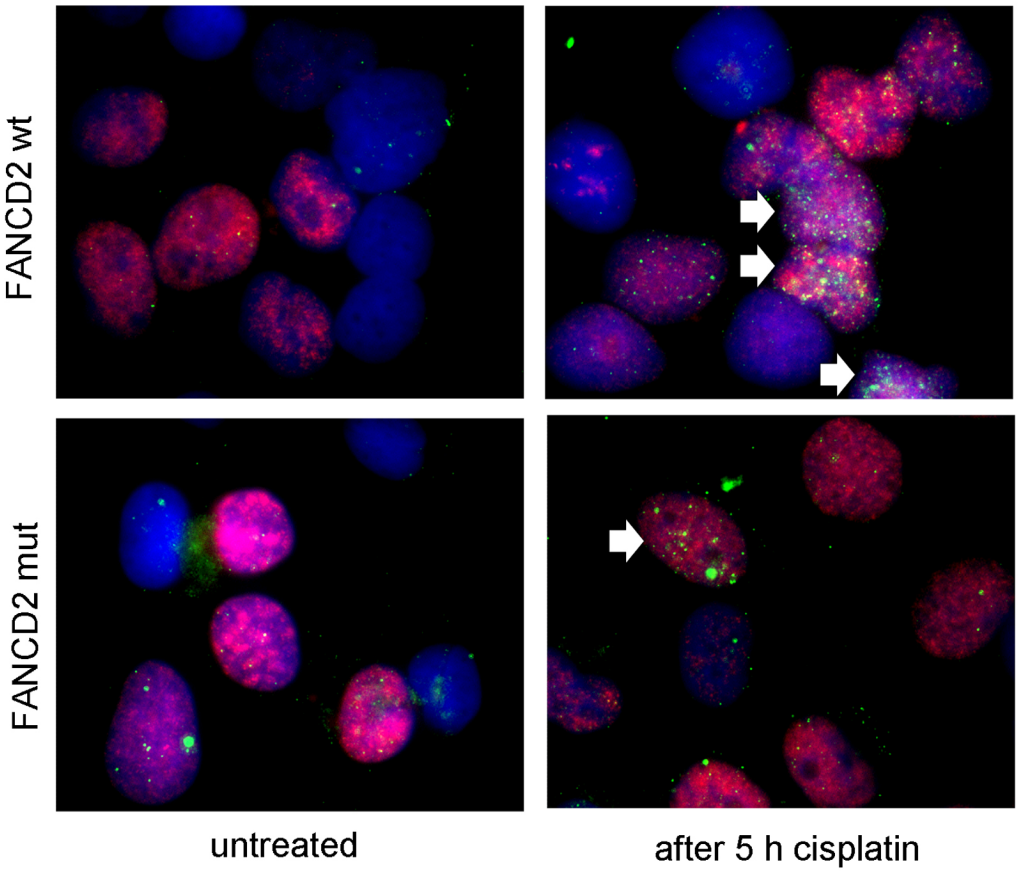
ice for 5 minutes, followed by fixation with 2% paraformaldehyde at room temperature for 20 minutes and 100% methanol at –20°C for 10 minutes. Cells were exposed to primary anti-pATM pS1981 antibody (see above) and anti-PCNA (Rabbit polyclonal antibody, Abcam ab2426) at 1:200 dilution in 2% BSA, 0.1% Triton X-100 in PBS, for 2 hours at room temperature. The percentage of nuclei with either PCNA or p-ATM or the combination of both was scored at the time points indicated. At least 100 nuclei were counted per data point. Bars represent the mean of 2 biological replicates.

FIGURE S5. Analysis of Fanconi Anemia (FA) pathway function in H1299 cells. A, Whole cell lysates from exponentially growing NSCLC cells were subjected to Western blotting with anti-FANCF rabbit polyclonal antibody (Sigma-Aldrich, SAB1101098) at a 1:500 dilution. Standard beta-actin (Sigma-Aldrich) was used as a loading control. B, Exponentially growing cell lines were exposed to 40 ng/mL MMC for 24 hours. Whole cell lysates were subjected to Western blotting with anti-FANCD2 (FI17) mouse monoclonal antibody (Santa Cruz, sc 20022) at 1:100 dilution. In order to optimize separation of the damage-induced mono-ubiquitinated form of FANCD2 (L) from the unmodified form (S), a 3-8% TrisAcetate gradient gel (BioRad) was used. C, Western blot for RAD51 using anti-Rad51 mouse monoclonal antibody (GeneTex, GTX70230) at 1:1,000 dilution.

FIGURE S6. Translation of cell line findings to tumor tissues. A, Correlation of Rad51 foci formation with clonogenic cell survival after treatment with 8 mM cisplatin. Upper panel correlates survival with "induced" RAD51 foci, i.e., fraction of nuclei with at least 15 RAD51 foci following cisplatin treatment minus fraction of nuclei with at least 15 RAD51 foci in untreated cells. Lower panel shows lack of correlation of survival with "baseline" fraction of nuclei with at least 15 RAD51 foci without cisplatin treatment. B, Representative images demonstrating induction of γ-H2AX foci in NSCLC explants following 5 hours after irradiation (IR) with 10 Gy or treatment with 8 μM cisplatin. Arrows

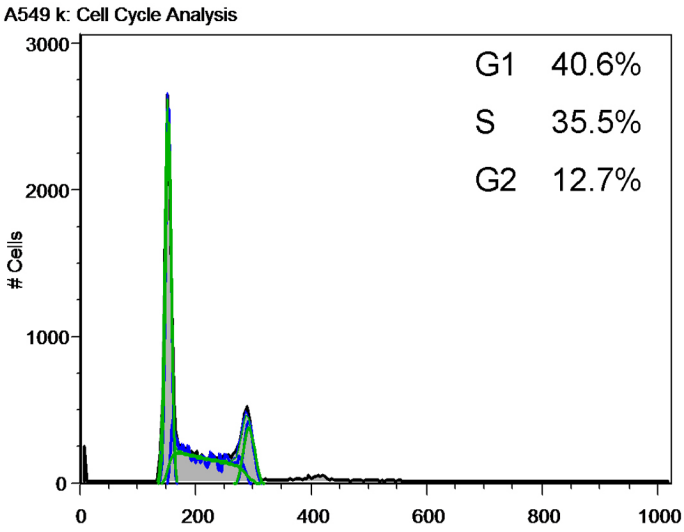
indicate foci, which are less frequent and more difficult to discern after cisplatin than after radiation treatments. C, Estimation of the fraction of cells in S-phase. Upper panel, the fraction of nuclei with FANCD2 foci (which almost exclusively form in S-phase) and PCNA is shown for A549 cells. Approximately 50% of cells are in S-phase. Lower panel, semi-quantification of nuclei with at least 2 FANCD2 foci in untreated NSCLC explants. Percentages represent averages based on 7-10 random images and 200-400 nuclei.

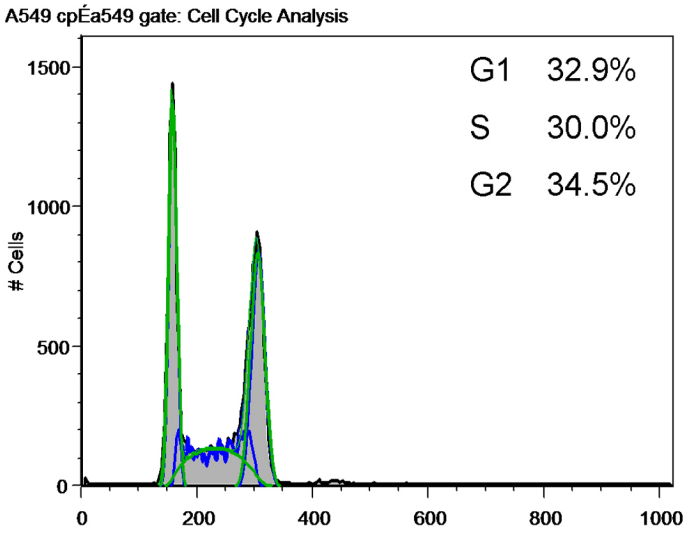
Suppl. Fig. S1



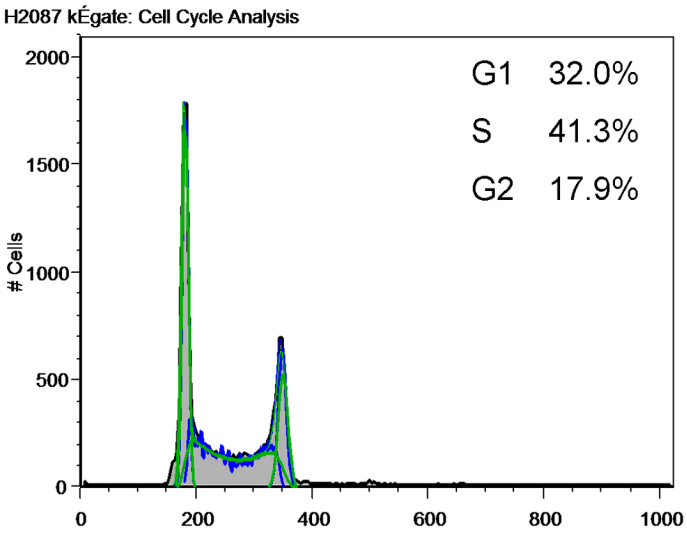
Suppl. Fig. S2

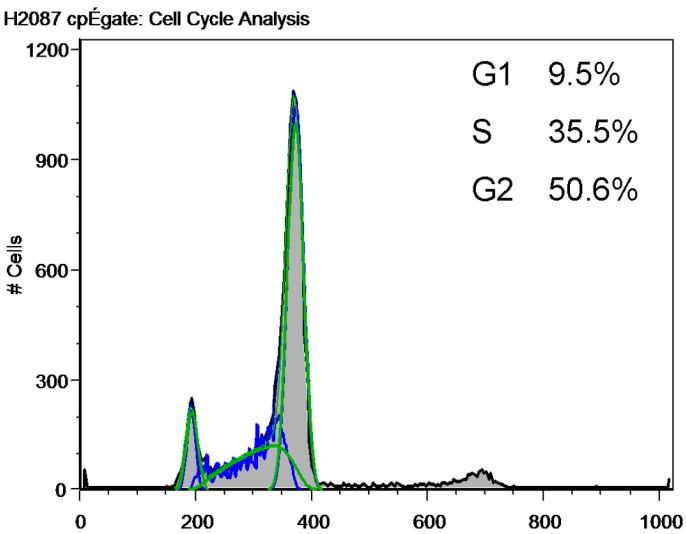
A549



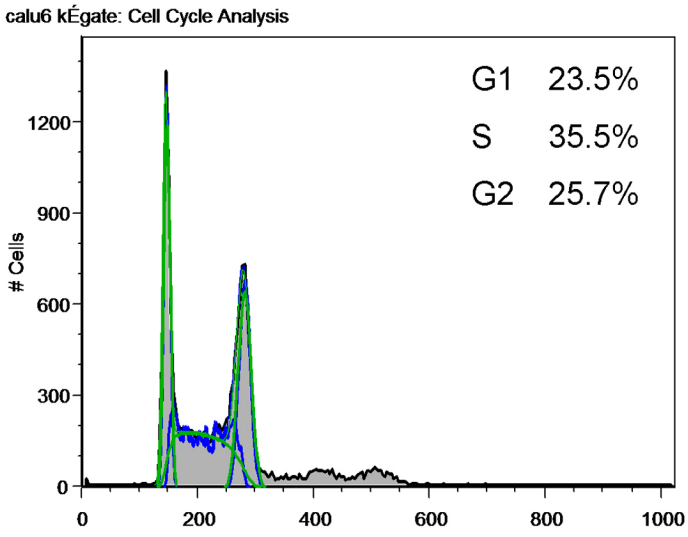


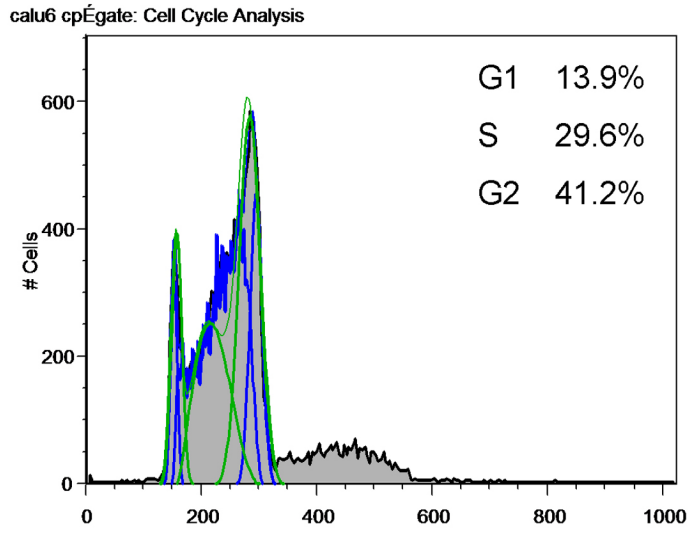
H2087





Calu-6

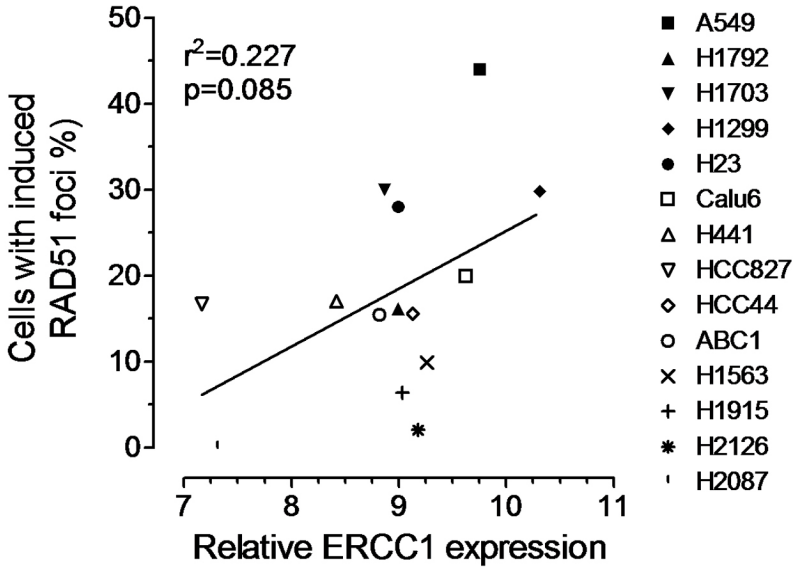




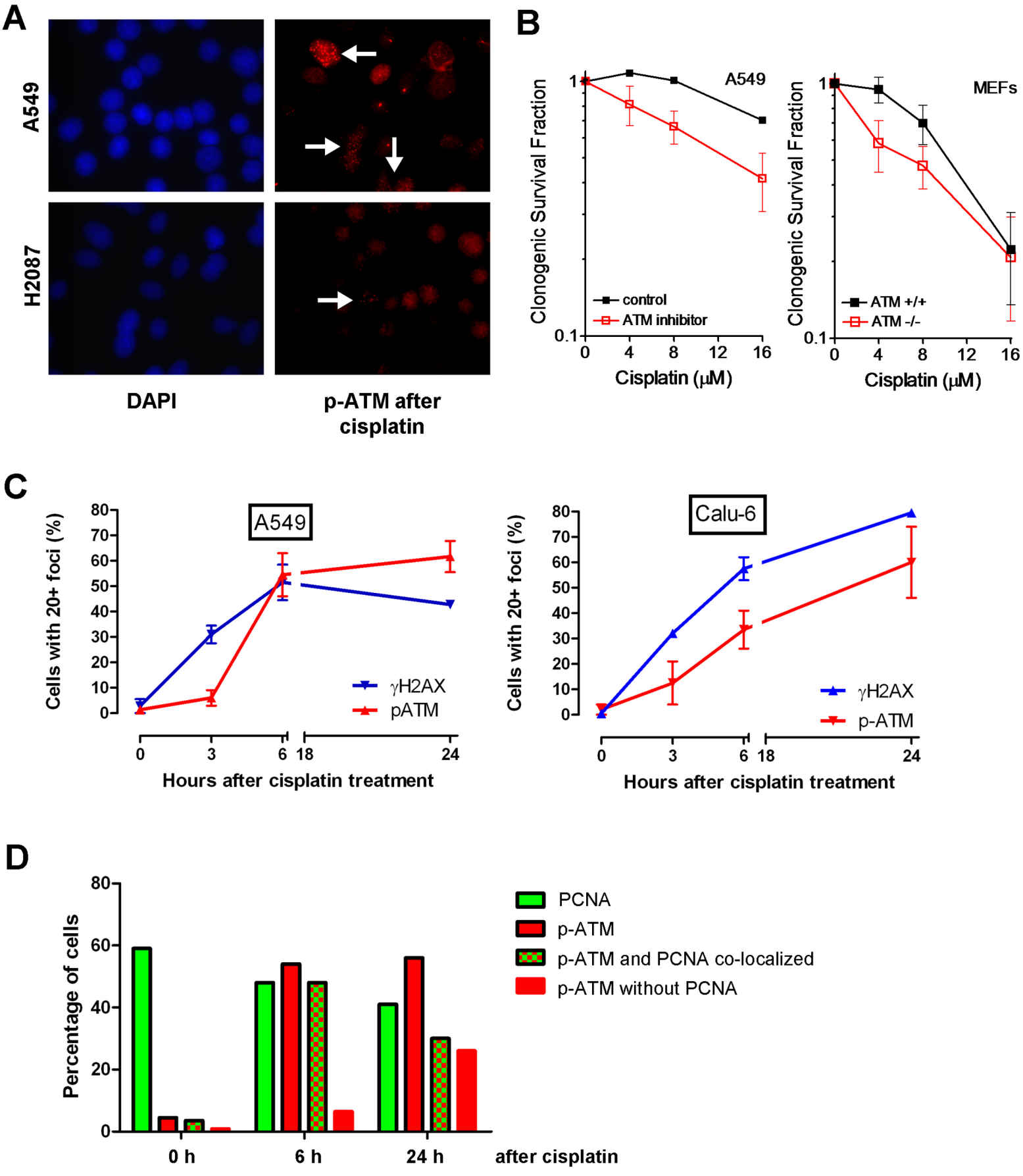
untreated

cisplatin

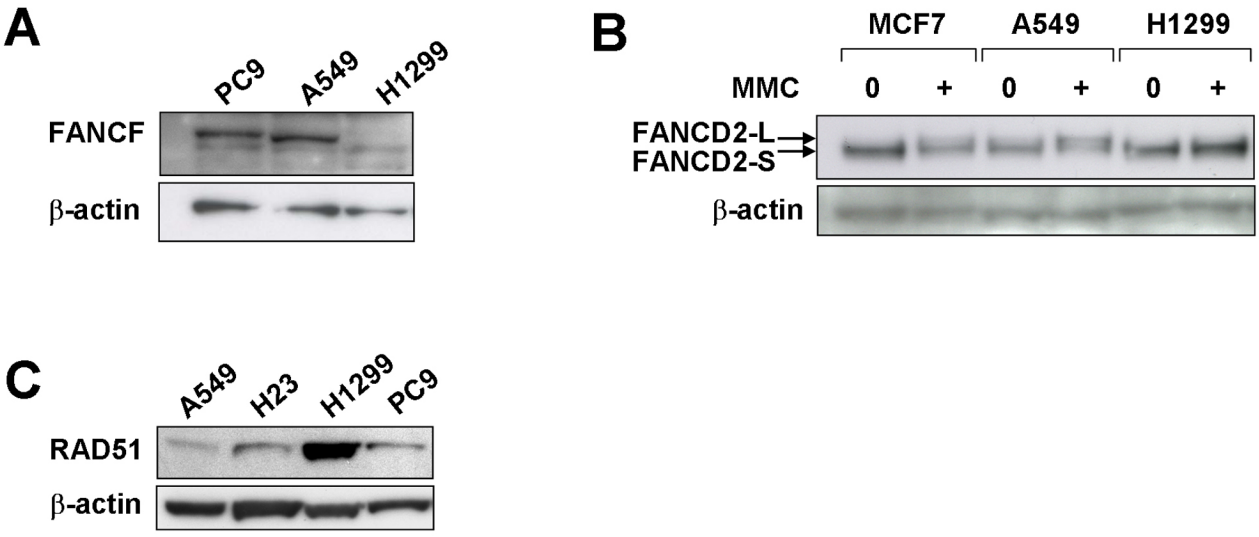
Suppl. Fig. S3


r2=0.227
p=0.085
Cells with induced RAD51 foci %)
Relative ERCC1 expression
0
10
20
30
40
50
7
8
9
10
11
A549
H1792
H1703
H1299
H23
Calu6
H441
HCC827
HCC44
ABC1
H1563
H1915
H2126
H2087

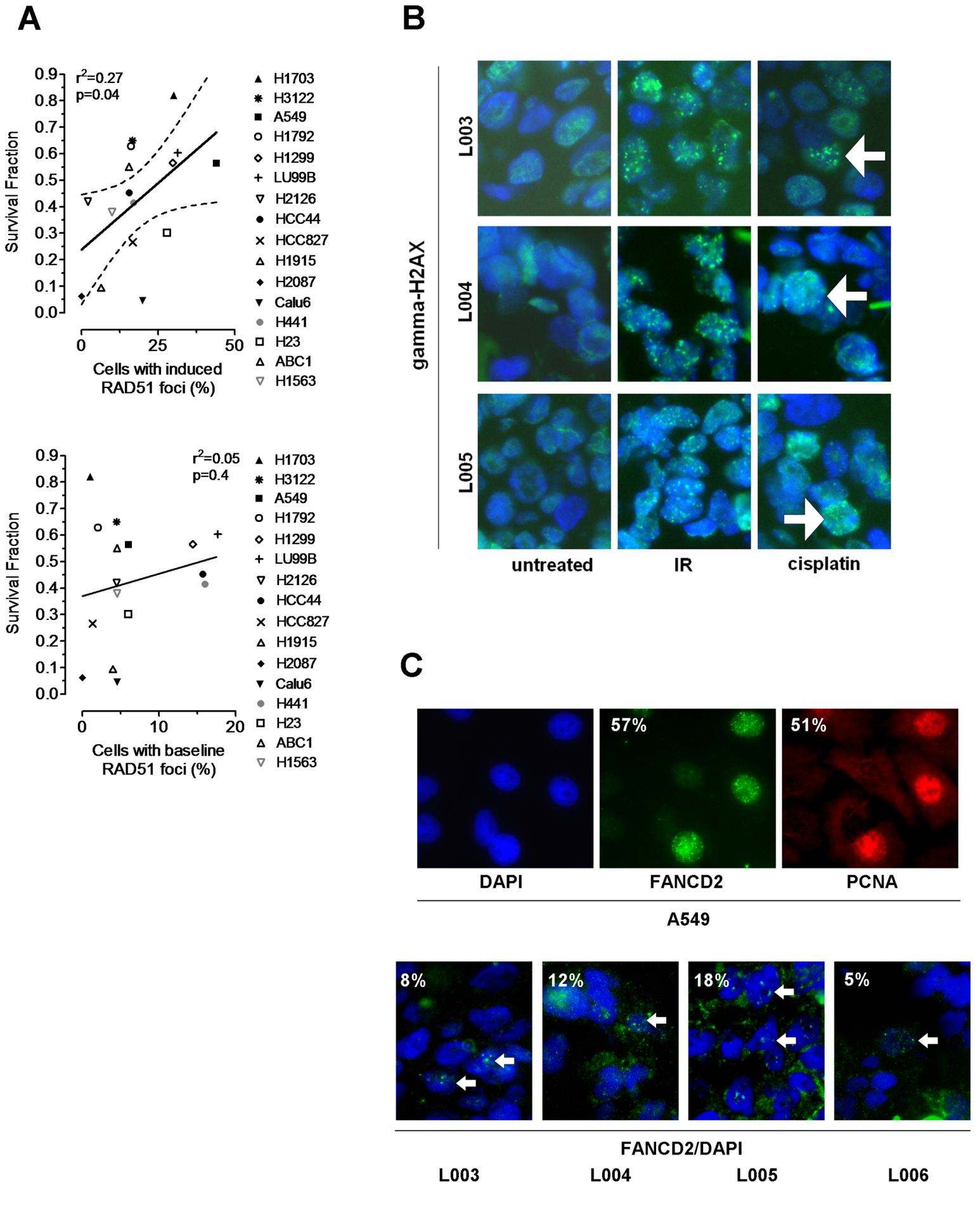
Suppl. Fig. S4


A
A549
H2087
DAPI
p-ATM after cisplatin
B
Clonogenic Survival Fraction
A549
control
ATM inhibitor
Cisplatin (μM)
MEFs
ATM +/+
ATM -/-
C
A549
Calu-6
Cells with 20+ foci (%)
γH2AX
pATM
p-ATM
Hours after cisplatin treatment
D
Percentage of cells
PCNA
p-ATM
p-ATM and PCNA co-localized
p-ATM without PCNA
0 h
6 h
24 h
after cisplatin

Suppl. Fig. S5


A
PC9
A549
H1299
FANCF
β-actin
B
MCF7
A549
H1299
MMC
0
+
0
+
0
+
FANCD2-L
FANCD2-S
β-actin
C
A549
H23
H1299
PC9
RAD51
β-actin

Suppl. Fig. S6


A
r²=0.27
p=0.04
Survival Fraction
Cells with induced RAD51 foci (%)
H1703
H3122
A549
H1792
H1299
LU99B
H2126
HCC44
HCC827
H1915
H2087
Calu6
H441
H23
ABC1
H1563
r²=0.05
p=0.4
Cells with baseline RAD51 foci (%)
B
gamma-H2AX
L003
L004
L005
untreated
IR
cisplatin
C
57%
51%
DAPI
FANCD2
PCNA
A549
8%
12%
18%
5%
FANCD2/DAPI
L003
L004
L005
L006