Supplemental data

Figure S1

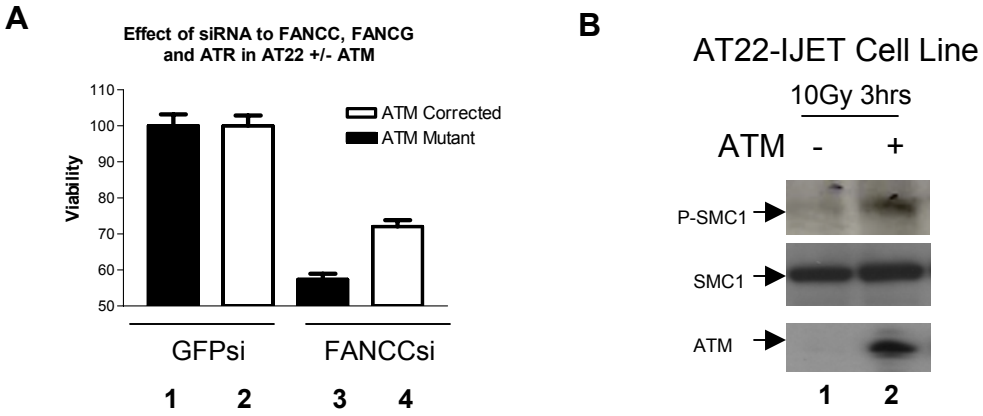


Figure S2

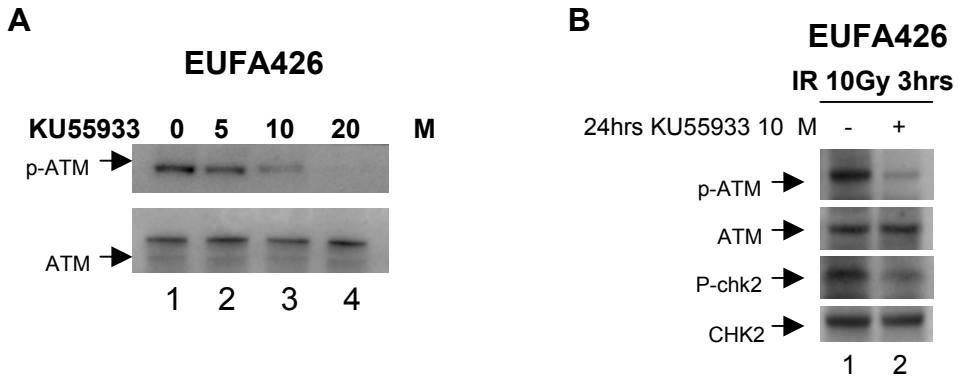

A
EUFA426
KU55933 0 5 10 20 M
p-ATM
ATM
1 2 3 4
B
EUFA426
IR 10Gy 3hrs
24hrs KU55933 10 M - +
p-ATM
ATM
P-chk2
CHK2
1 2

Figure S3

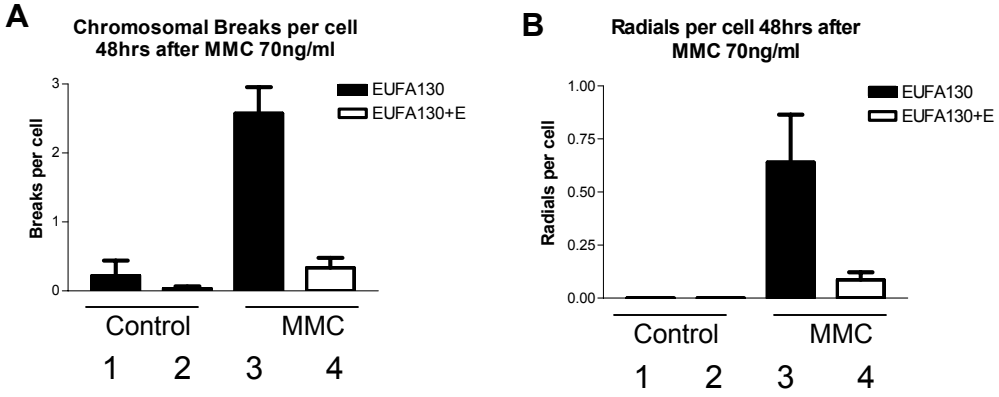
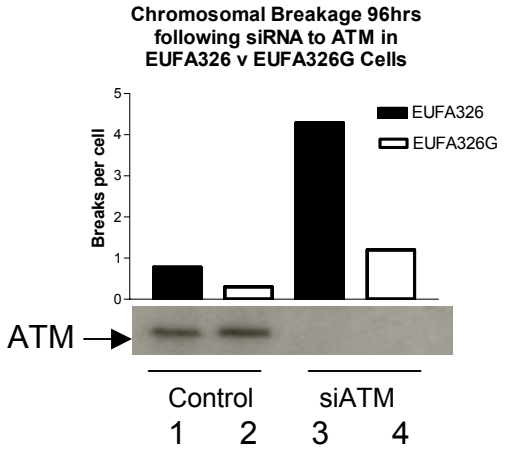

A
Chromosomal Breaks per cell
48hrs after MMC 70ng/ml
Breaks per cell
3
2
1
0
EUFA130
EUFA130+E
Control
MMC
1
2
3
4
B
Radials per cell 48hrs after
MMC 70ng/ml
Radials per cell
1.00
0.75
0.50
0.25
0.00
EUFA130
EUFA130+E
Control
MMC
1
2
3
4

Figure S4


Chromosomal Breakage 96hrs following siRNA to ATM in EUFA326 v EUFA326G Cells
Breaks per cell
0
1
2
3
4
5
EUFA326
EUFA326G
ATM
Control
siATM
1
2
3
4

Supplemental Figure Legends

Figure S1: The Combined Loss of the FA pathway and *ATM* Function is Toxic to Cells.

A: The AT22-IJET and AT22-IJET-ATM cell lines were treated for 96hrs with control GFP siRNA (lanes 1 and 2) or siRNA targeting *FANCC* (lanes 3 and 4). Viability is given as a percentage of a no siRNA treatment control for each cell line with the SEM from 3 experiments. Lanes 1 and 3 of the Western blot and bars 1 and 3 of the graph represent the *ATM* deficient AT22-IJET cell line, lanes 2 and 4 of the Western blot and bars 2 and 4 of the viability graph represent AT22-IJET-ATM corrected cell line.

B: A Western blot demonstrating functional correction of the AT22-IJET cell line with exogenously expressed ATM. Lane 1 represents the ATM-deficient AT22-IJET line, lane 2 the isogenic corrected cell line. Both cell lines were treated with 10Gy ionizing radiation and lysates blotted for ATM expression and ATM-mediated phosphorylation of SMC1 at serine 957 (Abcam).

Figure S2: KU55933 effectively inhibits ATM in human fibroblast cell lines

A: EUFA 426 cells were treated for 24hrs with KU55933 at concentrations of 5 M (lane 2), 10 M (lane 3) and 20 M (lane4). Lane 1 represents a DMSO only control treatment. Lysates were probed by Western blotting for autophosphorylation of ATM.

B: EUFA426 cells were treated for 24hours with KU55933 10 M (lane 2) or DMSO only (lane 1) and then exposed to 10Gy ionizing radiation. 3hrs later lysates were extracted and probed for autophosphorylation of ATM and phosphorylation of CHK2 at threonine 68.

Figure S3: The EUFA130 FANCE Mutant Cell line is Effectively Corrected by Expression of the Wild type FANCE Gene.

A: Bars 1 and 3 represent the EUFA130 cell line, bars 2 and 4 the corrected EUFA130E cell line. Cells were exposed to mitomycin C 70ng for 48hrs (bars 3 and 4) or no treatment (bars 1 and 2). Chromosomal breaks were measured on metaphase spreads.

B: Bars 1 and 3 represent the EUFA130 cell line, bars 2 and 4 the corrected EUFA130E cell line. Cells were exposed to mitomycin C 70ng for 48hrs (bars 3 and 4) or no treatment (bars 1 and 2). Radial formation was measured on metaphase spreads.

Figure S4: The EUFA326 Cell Line Demonstrates Chromosomal Breakage 96hrs after Treatment with siRNA to ATM

Bars 1 and 3 of the graph and lanes 1 and 3 of the Western gel represent the EUFA326 cell line, bars 2 and 4 and lanes 2 and 4 represent the corrected EUFA326G cell line. Cells were treated with a GFP targeted siRNA (bars 1 and 2 and lanes 1 and 2) or ATM targeted siRNA (bars 3 and 4 and lanes 3 and 4). Chromosomal breakage was assessed on metaphase spreads at 96hrs. Lysates were extracted and probed for ATM expression by Western blotting at 96hrs.